Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Development and Characterization of Gamma-Oryzanol Loaded Folic Acid Conjugated Multi Walled Carbon Nanotubes

Jinendra Sardiya, Praveen Kumar Soni, Monika Rakse, Neeraj Sharma
Bhagwant University, Sikar Road, Ajmer, Rajasthan
School of Pharmacy, Sangam University, Bhilwara, Rajasthan
LCIT School of Pharmacy, Bilaspur, Chhattisgarh
IPS Academy College of Pharmacy, Indore, M.P.

Address for correspondence
Dr. Neeraj Sharma
IPS Academy College of Pharmacy
Indore, M.P.
Mail ID:-neerajsharma236@gmail.com

Cite this paper as: Jinendra Sardiya, Praveen Kumar Soni, Monika Rakse, Neeraj Sharma (2024). Development and Characterization of Gamma-Oryzanol Loaded Folic Acid Conjugated Multi Walled Carbon Nanotubes. *Frontiers in Health Informatics*, *Vol.13, No.8*, 6860-6868

Abstract
Ancient cultures placed a great deal of importance on plants in their folklore. Plants have been used for more than 5000 years as remedies in addition to food and spices. It is estimated that between 70 and 95 percent of people in poor nations still consume. These days, plants containing important compounds that have therapeutic or helpful effects in the treatment and prevention of a wide range of human and animal disorders are referred to as medical herbs. Herbal goods have been used in addition to conventional medications as supplementary therapies. MWCNTs were functionalized one after the other to create γ-OZ/FA-MWCNTs. In phosphate buffer solutions (PBS) with a pH of 7.4, γ-OZ was physically loaded onto virgin MWCNTs and FA-MWCNTs. The lung epithelial cancer cell line "A-549" was used to evaluate the entrapment efficiency, in vitro release, and ex vivo tests. In both the pristine and FA-MWCNT formulations, γ-OZ was successfully incorporated, with the FA-MWCNTS formulation demonstrating the greatest γ-OZ trapping at 86.5 1.5%. The in vitro release of γ-OZ from γ-OZ/FA-MWCNTs at 7.4 (PBS) was investigated in the study, revealing a faster initial release followed by a sustained release. Additionally, γ-OZ/FA-MWCNTs was found to have a cytotoxic impact when used on the "A-549" lung epithelial cancer cell line.

Key words: - Gamma Oryzanol, Functionalization, Folic acid,

Introduction

Ancient cultures placed a great deal of importance on plants in their folklore. Plants have been used for more than 5000 years as remedies in addition to food and spices. It is estimated that between 70 and 95 percent of people in poor nations still consume. These days, plants containing important compounds that have therapeutic or helpful effects in the treatment and prevention of a wide range of human and animal disorders are referred to as medical herbs. Herbal goods have been used in addition to conventional medications as supplementary

therapies. These items include plant extracts, dry powder, and pieces of plants, fungi, and algae (Robinson & Zhang et al., 2011). Rice bran oil is a valuable byproduct of the rice milling sector that is growing in commercial significance due to its many health benefits. Despite having a comparable fatty acid profile to other oils, rice bran oil possesses hundreds of bioactive chemicals that give it its anti-cancer, cholesterol-lowering, and antioxidant properties (Sohail & Rakha et al., 2016). Over the past few years, these features have been the subject of much research. The cytotoxic effect of IR64 rice bran oil was demonstrated by Dapar et al. (2013) against A549 lung adenocarcinoma epithelial cells, an NSCLC cell line harboring KRAS mutations. According to this research, a few of the bioactive phytochemicals found in rice bran oil may be suitable options for treating or preventing NSCLC (Dapar & Garzon et al., 2013). The pericarp, seed coat, nucellus, aleurone, and soft germ of a rice seed are all included in rice bran, which makes about 6% of a rice kernel. The oil content of bran ranges from 15 to 20%, of which 4% is unsaponifiable substance. This is a pretty high percentage when considering other vegetable oils.Tocopherols and tocotrienols, which are derivatives of vitamin E, and γ-oryzanol (1–3% w/w) make up the majority of the unsaponifiable substance. The bioactive ingredient in rice bran oil with the most advantageous qualities is γ-Oryzanol. Although it was first believed to be a single ingredient, it is actually a complicated mixture of plant sterols and various ferulate esters of triterpene alcohols. The most prevalent molecules in γ-oryzanol are 24-methylenecyloartanyl ferulate (24-mCAF), cycloartenylferulate (CAF), campesterylferulate (CMF), and β-sitosterylferulate (β-SF), which account for approximately 94% of the total mass (Patel & Naik et al., 2001; Britz & Prasad et al., 2007;Kim et al., 2015). The bulk of the rice bran extract is gamma-oryzanol, which accounts for 2% of its total. Phytosterylferulates make up the majority of rice bran oil. Rice bran oil has been shown in numerous studies to have several health benefits, including its capacity to reduce plasma cholesterol, inhibit platelet aggregation, and possess unique antioxidant qualities. Traditional Japanese medicine has employed rice bran oil to treat gastrointestinal disorders, improve skin capillary circulation, encourage growth, and mitigate the symptoms of menopause. Chemotherapy, cancer, and radioprotective effects have all been related to high antioxidant activity (Ismail M et al., 2010; Valantina et al., 2010). Among the materials discovered in the last 30 years, the properties of carbon nanotubes have attracted intense interest from the scientific community as well. Finding novel and efficient drug delivery methods is a crucial problem that will always be of interest. The general goal of a drug delivery system is to enhance a medication molecule's pharmacological and therapeutic characteristics. Due to their ability to penetrate cells, f-CNTs may be used as carriers for the delivery of minute therapeutic molecules. Iijima et al. (1993) demonstrated that rolling up numerous graphene sheets to create concentric cylinders (multi-walled CNT) or a single layer of graphene sheet (single-walled CNT) are two innovative ways to produce CNT. Commercially available as-produced CNT with varying degrees of purity and distinct structural features is available for both SW and MW (Bethune et al., 1993).

Due to the advancement of efficient methods for chemically modifying CNT, soluble CNT may be produced, making it particularly promising for a variety of biological applications, such as drug delivery (Bianco et al., 2005 ;Tian et al 2007). For the modification of CNT, two functionalization techniques are commonly used. Strong acids can be used to oxidize CNT, which reduces their length and produces carboxylic groups that make them more soluble in

aqueous solutions (Kostarelos et al., 2005). As an alternative, CNTs become soluble in water through addition reactions to their tips and external walls (Liu et al., 1998). For carbon nanotubes to be considered biocompatible, they must be soluble in physiological solutionsAdditionally, functionalized carbon nanotubes (f-CNT) may be used to bind peptides, proteins, nucleic acids, and other pharmaceutical substances to a variety of active molecules.

We produced γ-OZ/FA-MWCNTs loaded with folic acid conjugated multiwalled carbon nanotubes (γ-OZ/FA-MWCNTs) as a method of enhancing gamma-oryzanol availability. Fourier transform infrared (FT-IR) spectroscopy was used to further characterize the produced γ-OZ/FA-MWCNTs. In the end, the suggested mixture underwent cytotoxicity tests, in-vitro release profile analysis, and entrapment efficiency analysis.

Material and method

Multi-walled carbon nanotubes (MWCNTs) produced by chemical vapour deposition (CVD) with purity of 99.3 %, diameter × length 110-170 nm ×5-9 µm and melting point 3652-3697° C, was purchased from Platonic Nanotech Private Limited, Jharkhand, India. Gamma-oryzanol was purchased from A. P. organic Pvt Ltd. Punjab, India. All reagent like Ethylene diamine, N,N-dimethyl formamide, Tetrahydrofyran(THF), Dimethylsulphoxide (DMSO), N-Hydroxysuccinimide (NHS) and folic acid (FA) were purchased from commercial supplier and used as received.

Purification of carbon nanotubes

MWCNTs purification based on the concepts of metallic impurity dissolving by acids and selective oxidation, where carbonaceous impurities oxidize more quickly than CNTs (Peng-Xiang et al., 2008). Catalytic and amorphous impurities were eliminated from the unpurified MWCNTs by acid treatment. A certain amount of unpurified MWCNTs was obtained and then placed into strong hydrochloric acid for five hours while being stirred magnetically. The mixture was then filtered using a 0.45 µm polytetrafluoroethylene (PTFE) filter (Sigma Aldrich, USA).In order to eliminate the amorphous carbon, acid-purified MWCNTs were placed in an oven and kept there for 30 minutes at 530°C (Li J & Zhang Y et al., 2006; Kesharwani et al., 2012).

Cutting and Carboxylation of purified MWCNTs

The type of polymer to be reinforced determines which chemical group ought to be bonded to the nanotubes. Several authors (Goyanes et al., 2007). have suggested that the presence of carboxylic acid groups on the surface of carbon nanotubes (CNTs) is a common path towards accomplishing this goal because these groups can be the source of a wide range of chemical reactions.

The concentrated H2SO4 and HNO3 (3:1) ratio was used to oxidize the purified MWCNTs in a sonication tube for 15 minutes. After sonication, the prepared suspension was put into a round-bottom flask (RBF) and magnetically stirred for four and twelve hours at 60 ± 2°C. It was then filtered and cleaned with deionized water. Lastly, the black solid residue was vacuum-dried for an entire night at room temperature (RT) (Li J & Zhang Y et al., 2006; Kesharwani et al., 2012).

Acylation and amidation of carboxylated MWCNTs

For 24 hours, carboxylated MWCNTs were continuously stirred at 70 ± 2°C while 30 mL of thionyl chloride (SOCl2) and dimethyl formamide (DMF) were added in a 20:1 ratio.

To remove extra thionylchloride, the resultant suspension was filtered and five times treated with anhydrous tetrahydrofuran (THF). An oven with a vacuum was used to dry the residual solid. For two days, at 100 ± 2°C, 10 mL of ethylene diamine solution (EDA) was reacted with 20 mg of acyl-chlorinated MWCNTs. To get rid of extra diamine, MWCNTs were cooled to room temperature and then five times in ethanol. Ultimately, the black solid residue was vacuum-dried for an entire night at room temperature (Li J & Zhang Y et al., 2006; Kesharwani et al., 2012).

Conjugation of folic acid to amine modified MWCNTs

A beaker containing 25 ml of methanol was filled with known quantity of amine-modified MWCNTs, and 500 milligrams of folic acid was added to the mixture. After processing the reaction for five days at room temperature while stirring continuously, acetone was added to produce a yellow precipitate. Filtered and dried, folate conjugated MWCNTs (f-MWNCTs) were analyzed using techniques such as FT-IR, H-NMR, and XRD (Neelesh et al., 2013;Neelesh et al., 2015;Rakesh et al., 2008).

Loading of drug (Gamma-oryzanol) in MWCNTs

Evaluation of FA conjugated MWCNTs' drug-holding and drug-release capabilities in release media was part of their characterization process. To help in drug encapsulation, twenty milligrams of gamma-oryzanol and ten milligrams of f-MWCNTs (2:1 ratio) dispersed in phosphate buffer saline (pH=7.4) were combined, and the mixture was agitated overnight. The drug-loaded f-MWCNTs were separated from the solution by ultracentrifugation after encapsulation. By measuring the amount of γOZin supernatant with a UV spectrophotometer (Shimadzu 1601, Japan), the amount of γOZentrapped in f-MWCNTs based systems was determined independently. A similar process was used with pristine MWCNTs (Jitendra et al., 2016).

***In vitro* Drug release studies**

Under physiological conditions (PBS; pH 704), the *in vitro* release of medication from two different formulations (pristine MWCNTs and f-MWCNTs) was determined. For release investigations, the dialysis membrane (MWCO; 2000 Da) was chosen. After inserting five milligrams of the formulation into the dialysis sac and sealing it from the outside, it was suspended in 100 milliliters of aqueous receptor release medium right away. The *in vitro* drug release experiment was performed in the receptor compartment at 37±0.5°C with continual stirring under tight sink conditions. At each of the prearranged intervals, one milliliter of the sample was taken out and replaced with an equivalent volume of new medium. Drug was measured using a spectrophotometer (UV/Vis Shimadzu 1601, Japan) at 314 nm after the proper dilutions.

Cytotoxicity studies

An environment of 37 ± 0.5°C, humidified with 5% CO2, was used to culture the lung epithelial cancer cell line A-549 in RPMI-1640 medium. The medium was supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, and antibiotics (50 IU/mL penicillin and 50 µg/mL streptomycin) (Agarwal A & Gupta U et al., 2009).

A non-radioactive colorimetric assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was utilized in the investigation to measure the inhibition of cell growth. By incubating A-549 cells with the γOZ/FA-MWCNTs formulation in vitro, cytotoxicity was assessed. The A-549 cells were cultivated to 80% confluence in 37 ± 0.5°C

humidified incubator with 5% CO_2 environment using RPMI-1640 media supplemented with 10% fetal calf serum, 2 mM glutamine, 50 IU/mL penicillin, and 50 µg/mL streptomycin. In 96 tissue culture plates with a flat bottom, A-549 cells were planted at a density of 2×10^5 cells/mL and incubated for a full day. The cultivated cells were subjected to sequential treatments of γOZ/FA-MWCNTs suspension at concentrations of 10–100µg/mL, and were then incubated for 24 hours in a controlled environment.Then, each well was filled with MTT solution dissolved in phosphate buffer solution (PBS; pH 7.4), and the wells were incubated for 8 hours at 37 ± 0.5°C to allow the viable cells to convert the MTT to purple formazan crystals. Using the following equation, cell viability was assessed in an Elisa plate reader at 570 nm. For every sample in the MTT experiment, three replicates were read, and the mean value was utilized to determine the final result, which was significant ($p < 0.05$).

Stability studies

For five weeks, the suspension of γOZ/FA-MWCNTs was kept in tightly sealed amber and colorless glass vials at four degrees Celsius, room temperature (25 degrees Celsius), and thirty-five degrees Celsius in an oven with a thermostat. The suspension was periodically checked for changes in precipitation, crystallization, color, consistency, and turbidity. The acquired data was utilized to analyze any degradation, both chemical and physical, and to recommend the necessary storage conditions. Monitoring drug release from the γ-OZ/FA-MWCNTs suspension following storage under various circumstances allowed for the determination of drug leakage as well. To do this, a weekly sample of 2 ml was taken, suitably diluted, and subjected to an HPLC method of analysis. This process was repeated every week for a maximum of five weeks.

Result and Discussion

The MWCNTs were acquired from Jharkhand, India's Platonic Nanotech Private Limited. There is only one peak in the purified pristine MWCNTs' FT-IR spectra (figure 1A), indicating that there are no impurities present. Folic acid was coupled with amine-terminated MWCNTs (figure 1B). The aromatic carbon-hydrogen bending peak, as well as the aromatic carbon=carbon bending and stretching peak, are visible in the FT-IR spectra of FA conjugated MWCNTs (f-MWCNTs).

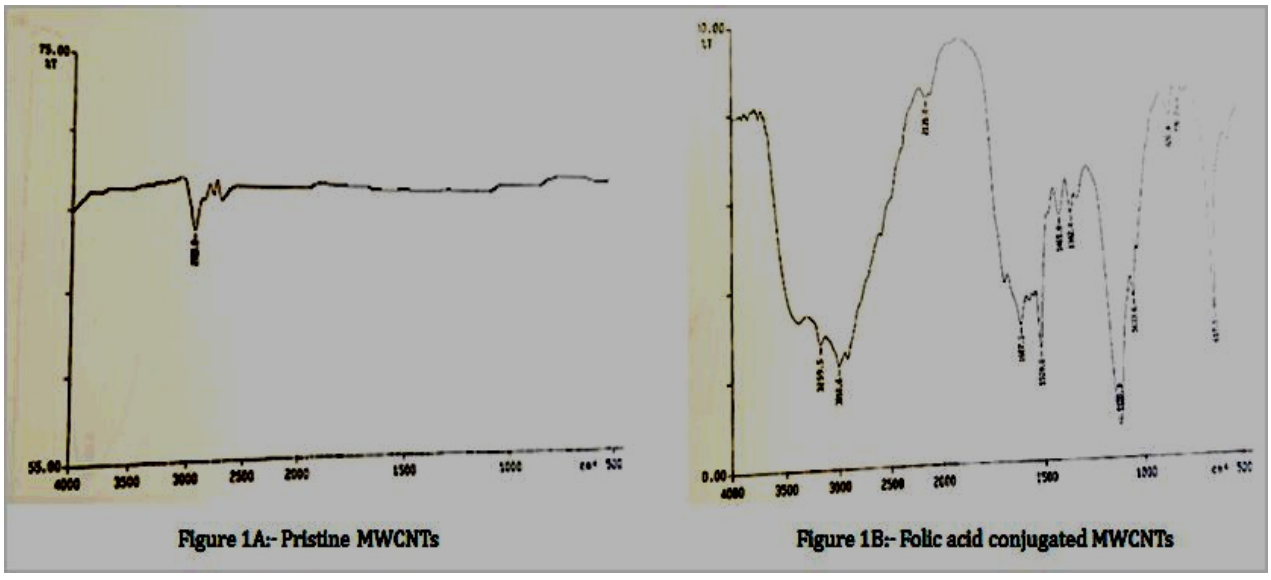

Figure 1:- FT-IR spectrum

Using the equilibrium dialysis approach, Gamma-oryzanol (γ-OZ) was loaded into pristine and conjugated folic acid-containing MWCNTs. The drug loading experiment was carried out in a phosphate buffer saline pH 7.4 dispersion medium at room temperature. For folic acid conjugated MWCNTs (FA-MWCNTs), a higher drug loading of 86.5 ± 1.5% was noted. On the other hand, pure MWCNTs showed lower drug loading, at 62.4 ±1.9% (figure 2). Phosphate buffer saline pH 7.4 was used as the receptor media in the equilibrium dialysis method, which was used to measure the release of γ-OZfrom folic acid linked MWCNTs and pristine MWCNTs. In a 24-hour period, the drug release from pristine MWCNTs was observed to be 69.26±1.5%, whereas folic acid conjugated MWCNTs released 58.96±68% (figure 3).

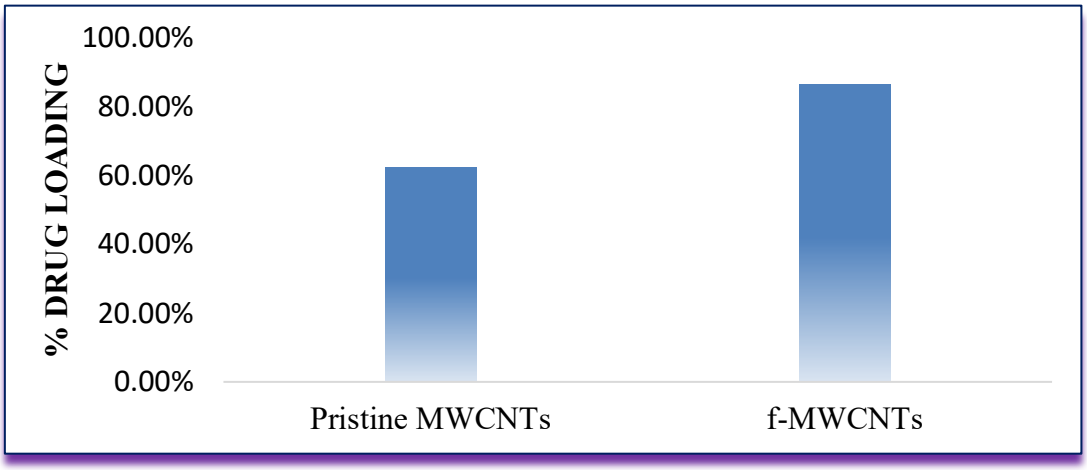

Figure 2:- Drug loading efficiency (n=3)

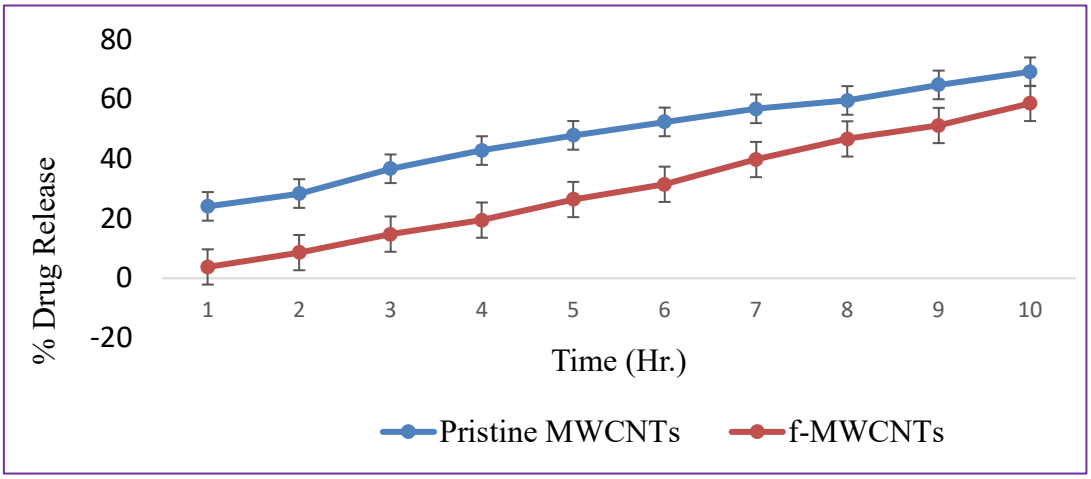

Figure 3:- % Drug Release of γ-OZ in Pristine & Folic acid attached MWCNTs (n=3)

The MTT test was used to evaluate the cytotoxicity of γ-OZ/FA-MWCNTs on A-549 cells at equivalent dosages ranging from 10 to 100µg/ml. The results unequivocally demonstrated that γ-OZ/FA-MWCNTs inhibited A-549 cells in a dose-dependent manner and were noticeably more cytotoxic at higher concentrations. Since γ-OZ/FA-MWCNTs target specific ligands, they exhibited a highly cytotoxic response. Figure 4 shows the uptake of γ-OZ by the lung epithelial cancer cell line in our investigations, which revealed dose-dependent uptake.

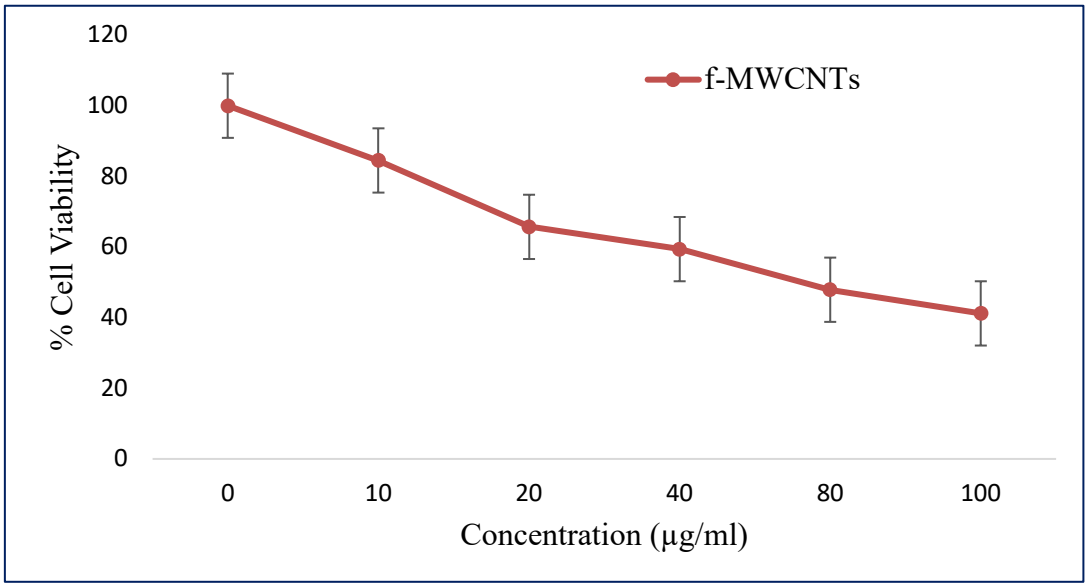

Figure 4:-% cell viability assay of γ-OZ/FA-MWCNTs formulation (n=3)

Conclusion

CNTs are a novel type of carriers that can be used to deliver medications in a target- and site-specific way. Because of their unique mechanical, chemical, and physical characteristics, CNTs are an effective biological carrier for anticancer medications. Owing to their distinct chemistry and hexagonal carbon atom arrangement, carbon nanotubes (CNTs) offer multiple sites for covalent and noncovalent functionalization with therapeutically active molecules. This suggests that CNTs may be used as nanocarriers to deliver therapeutic agents to specific cancer cell targets. These functionalized CNTs have a strong inclination to cross cell membranes through independent or dependent mechanisms on endocytosis. The formulation of γ-OZ-loaded folic acid-MWCNTs demonstrated effective γ-OZ release to the intended site with an enhanced therapeutic margin of safety, it may be inferred.

References

1. Robinson, MM., Zhang, X. (2011). Traditional Medicines: Global Situation, Issues and Challenges. Geneva: *World Health Organization*. https:// DOI: 10.4236/oalib.1105500.
2. Sohail, M., Rakha, A., Butt, M.S., Iqbal, M.J., Rashid, S. (2016) Rice bran nutraceutics: a comprehensive review. *Food Sci Nutr,* 1040 8398. https://doi:10.1080/10408398.2016.1164120.
3. Dapar, M., Garzon, J., Demayo, C. (2013). Cytotoxic activity and Antioxidant Potentials of hexane and Methanolextracts of IR64 Rice bran against Human Lung (A549) and Colon (HCT116) Carcinomas. *Int Res J Biological Sci.* (2) 19-23. https://www.isca.me/IJBS/Archive/v2/i5/3.ISCA-IRJBS-2013-006.pdf
4. Patel, N., Naik, SN. (2004). Gamma- oryzanol from rice bran oil – A review. *J. Sci. Ind. Res.* 63, 569-578. https://www.researchgate.net/publication/239785419
5. Britz, SJ., Prasad, PV., Moreau V., Allen, LH., Kremer, DF., Boote, KJ. (2007). Influence of growth temperature on the amounts of tocopherols, tocotrienols, and gamma-oryzanol in brown rice. *J Agric Food Chem*. 55, 7559-7565. https://DOI:10.1021/JF0637729

6. Kim, HW., Kim, JB., Cho SM. (2015). Characterization and quantification of γ-oryzanol in grainsof 16 Korean rice varieties. *Int J Food Sci Nutr*. 66, 166-174. https://doi: 10.3109/09637486.2014.971226.
7. Ismail, M., Al-Naqeeb, G., Mamat, WAA., Ahmad, Z. (2010). Gamma-oryzanol rich fraction regulates the expression of antioxidant and oxidative stress related genes in stressed rat's liver. *Nutr Metab*. 7, 23. https://doi: 10.1186/1743-7075-7-23
8. Valantina, SR., Sahayaraj, PA., Prema, AA. (2010). Antioxidant stability in palm and rice bran oil using simple parameters. *Rasayan J Chem*. 3, 44-50. https://rasayanjournal.co.in/vol-3/issue-1/7.pdf
9. Iijima, S., Ichihashi, T. (1993). Single-shell carbon nanotubes of 1-nm diameter. *Nature*. 363,603-605. https://www.nature.com/articles/363603a0.
10. Bethune, DS., Klang, CH., de Vries, MS., Gorman, G., Savoy, R., Vazquez, J., Beyers, R. (1993). Cobalt-catalysed growth of carbon nanotubes with single-atomic-layer walls. Nature 363,605-607. https://www.nature.com/articles/363605a0
11. Bianco, A., Kostarelos, K., Partidos, CD., Prato, M. (2005). Biomedical applications of functionalised carbon nanotubes. *Chem Commun*. 571-577. https://pubs.rsc.org/en/content/articlelanding/2005/cc/b410943k
12. Tian, H., Cronstein, BN. (2007). Understanding the mechanisms of action of methotrexate Bull. NYU Hosp. Jt. Dis., 2007, 65, (3), pp. 168 –173. https://pubmed.ncbi.nlm.nih.gov/17922664/
13. Kostarelos, K., Lacerda, L., Partidos, CD., Prato, M., Bianco, A. (2005). Carbon nanotube-mediated delivery of peptides and genes to cells:translating nanobiotechnology to therapeutics. J Drug Deliv Sci Technol. 15,4147.https://www.nanomedicinelab.com/wpcontent/uploads/2012/03/11thslide05.pdf
14. Liu, J., Rinzler, AG., Da,i H., Hafne,r JH., Bradley, RK., Boul, PJ., Lu, A., Iverson, T., Shelimov, K., Huffman, CB. (1998). *Fullerene pipes. Science*. 280,1253-1256. https://doi: 10.1126/science.280.5367.1253
15. Peng-Xiang, Hou., Chang, Liu., Hui-Ming, Cheng. (2008). Purification of carbon nanotubes, *Carbon*, 46, 2003-2025. https://doi.org/10.1016/j.carbon.2008.09.009.
16. Li, J., Zhang, Y. (2006). Cutting of multi walled carbon nanotubes. *Appl Surf Sci*, 252, 2944–2948. https://DOI:10.1016/j.apsusc.2005.04.039
17. Kesharwani, P., Ghanghoriya, R., Jain, NK. (2012) Carbon nanotubesexploration in cancer cell lines. Drug Discov Today, 17–18, 1023–1030. https://DOI: 10.1016/j.drudis.2012.05.003.
18. Goyanes, S., Rubiolo, GR., Salazar, A., Jimeno, A., Corcuera, MA., Mondragon, I. (2006). Carboxylation treatment of multiwalled carbon nanotubes monitored byinfrared and ultraviolet spectroscopies and scanning probe microscopy, *Diamond & Related Materials,* 16, 412–417. https://doi.org/10.1016/j.diamond.2006.08.021
19. Mehra, NK. Jain, NK. (2013). Development, characterization and cancer targeting potential of surface engineered carbon nanotubes, *J Drug Target*. 21(8), 745-58. https://doi: 10.3109/1061186X.2013.813028.
20. Mehra, NK. Jain, NK. (2015). One platform comparison of estrone and folic acid anchored surface engineered MWCNTs for doxorubicin delivery, *Mol Pharm.* 12(2), 630-43. https://doi: 10.1021/mp500720a.

21. Tekade, RK., Dutta, T., Tyagi, A., Bharti, AC., Das, BC., Jain, NK. (2008). Surface-engineered dendrimers for dual drug delivery: a receptor up-regulation and enhanced cancer targeting strategy, *J Drug Target*. 16(10),758-72. https://doi: 10.1080/10611860802473154.
22. Kayat, J., Mehra, NK., Gajbhiye, V., Jain, NK. (2016). Drug targeting to arthritic region via folic acid appended surface-engineered multi-walled carbon nanotubes, *J Drug Target*. 24(4), 318-27. https://doi: 10.3109/1061186X.2015.1077846.
23. Agarwal, A., Gupta, U., Asthana, A., Jain, NK. (2009). Dextran conjugated dendritic nanoconstructs as potential vectors for anti-cancer agent. *Biomaterials*, 30, 3588–3596. https://doi: 10.1016/j.biomaterials.2009.03.016.