Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2011: 3 (5) 34-47
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN 0974-248X
USA CODEN: DPLEB4

Effect of PEGylation on Multiwalled Carbon Nanotubes and Liposomes: A Comparative study

Chetana D. Modi[*1] and R. S. R. Murthy[2, 3]

[1]*Shree Krishna Institute of Pharmacy, Shankhalpur, Bechraji, Mehsana, Gujarat, India*
[2]*Department of Pharmaceutical Technology, Pharmacy Department, Faculty of Technology and Engineering, M. S. University, Vadodara, India*
[3]*ISF College of Pharmacy, G. T. Road, Moga, Punjab, India*

ABSTRACT

The covalent attachment of polyethylene glycol (PEG) polymer chain to the therapeutic moieties is known as PEGylation, which prolongs the residence time of drug in to the body by preventing RES uptake and renal clearance because of increased molecular weight. PEG compounds used for PEGylation may require targetable functional group at one end for covalent modification. Previously it has been proved that PEGylation of liposomes results in improved pharmacokinetic and bio-distribution of therapeutic drugs. PEGylation of carbon nanotubes makes them water dispersible and long circulating moieties. The present study is the investigation of effect of PEGylation on liposomes and multi-walled carbon nanotubes (MWNTs) loaded with methotrexate (MTX) anticancer drug. MWNTs-MTX conjugate was prepared by non-covalent functionalization and liposome-MTX conjugate was prepared by thin film hydration technique. PEGylation of both conjugates was done by DSPE-mPEG 2000. The comparison study of drug loading, particle size and in-vitro drug release profile from both conjugates represents effective results from MWNTs than liposomes. Higher drug loading on to carbon nanotubes was achieved (2.26 mg on 1 mg MWNTs) than liposomes. Particle size of both conjugates was preferred for IV administration i.e below 200 nm. In-vitro drug release from MWNTs was faster as compared to liposomes at acidic environment (pH 5.8), which represents pH at cancer cells. So they can be targeted for cancer treatment. It can be conclude that PEGylation of carbon nanotubes for methotrexate gives better effect on cancer than the PEGylated liposomes.

Keywords: Multiwalled Carbon Nanotubes, Methotrexate, DSPE-mPEG, Liposomes, PEGylation.

INTRODUCTION

PEG is the common abbreviation for polyethylene glycol – or, more properly, poly (ethylene glycol) – which refers to a chemical compound, composed of repeating ethylene glycol units (figure 1). Depending on how one chooses to define the constituent monomer or parent molecule

(as ethylene glycol, ethylene oxide or oxyethylene), figure 2 shows PEG compounds is also known as PEO (polyethylene oxide) and POE (polyoxyethylene). Purified PEG is most commonly available commercially as mixtures of different oligomer sizes in broadly or narrowly defined molecular weight (MW) ranges.

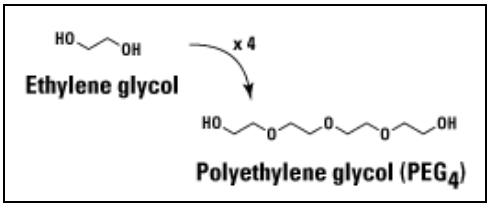
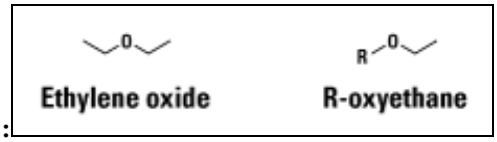


Figure 1: PEG unit.



:

Figure 2: PEO and POE unit.

Properties of Polyethylene Glycol

Poly (ethylene glycol) has mainly 3 chemical properties that make it especially useful in various biological, chemical and pharmaceutical settings:

- Non-toxic and non-immunogenic – can be added to media and attached to surfaces and conjugated to molecules without interfering with cellular functions or target immunogenicity
- Hydrophilic (aqueous-soluble) – attachment to proteins and other biomolecules decreases aggregation and increases solubility
- Highly flexible – provides for surface treatment or bioconjugation without steric hindrance

Certain experimental systems and assay platforms depend on the ability to alter the mass, solubility or other properties of proteins, immunogens, therapeutics, reaction vessels and other materials. PEGylation, the addition of ethylene glycol or ethylene oxide polymers, is a useful method of making these modifications. Covalent modification with PEG (also called PEO) groups requires PEG compounds that contain a reactive or targetable functional group at one end [1].

PEGylation is the process of covalent attachment of polyethylene glycol polymer chains to another molecule, normally a drug or therapeutic protein, which can help to meet the challenges of improving the safety and efficiency of much therapeutics. PEGylation is routinely achieved by incubation of a reactive derivative of PEG with the target macromolecule. The covalent attachment of PEG to a drug or therapeutic protein can "mask" the agent from the host's immune system (reduced immunogenicity and antigenicity); increase the hydrodynamic size (size in solution) of the agent which prolongs its circulatory time by reducing renal clearance. PEGylation can also provide water solubility to hydrophobic drugs and proteins. These physical and chemical changes increase systemic retention of the therapeutic agent. Also, it can influence the binding affinity of the therapeutic moiety to the cell receptors and can alter the absorption and distribution patterns. The choice of the suitable functional group for the PEG derivative is based on the type of available reactive group on the molecule that will be coupled to the PEG. For example, proteins have typical reactive amino acids, coupled to PEG, include lysine, cysteine, histidine, arginine, aspartic acid, glutamic acid, serine, threonine, and tyrosine. The N-terminal amino group and the C-terminal carboxylic acid can also be used as a site specific site by conjugation with aldehyde functional polymers.

PEGylation, by increasing the molecular weight of a molecule, can impart several significant pharmacological advantages over the unmodified form, such as [2]:

- Improved drug solubility.
- Reduced dosage frequency, without diminished efficacy with potentially reduced toxicity.
- Extended circulating life.
- Increased drug stability.
- Enhanced protection from proteolytic degradation.

Many studies and years of PEGylation development have given important theoretical and commercially useful results. The products already approved by the FDA are a clear demonstration of the usefulness of PEGylation in the improvement of therapeutic value of drugs. In particular, the increasing use of PEGylation was possible because of the availability of PEGs with different molecular weights and activation forms (mainly from Nektar Therapeutics) needed to respond to the various drug-modification requirements [3].

Protein and peptide drugs have short circulating half life. PEGylation can overcome these and other shortcomings by increasing the molecular mass of proteins and peptides and shielding them from proteolytic enzymes. It improves pharmacokinetics and show improved patient convenience and compliance [4].

An ideal PEG reagent fulfills at least the following criteria [5]:

- Monodispersity or at least a dispersity index close to 1.00, in order to assure a reproducible high quality.
- Availability of one single terminal reactive group for the coupling reaction, in order to avoid cross-linking between drug molecules.
- Non-toxic and non-immunogenic, biochemically stable linker.
- Branching for optimal surface protection.
- Options for site-specific PEGylation.

A liposome is a spherical vesicle with a membrane composed of phospholipids and cholesterol bilayer. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chains (like egg phosphatidyl-ethanolamine), or of pure surfactant components like DOPE (di-oleolyl-phosphatidyl-ethanolamine). PEGylated liposomes were initially developed with the primary goal of evading rapid clearance by the reticuloendothelial system, thus allowing them to remain in the circulation for prolonged periods after I.V. injection. This property of PEGylated liposomes has been shown to result in effective tumor targeting and therapeutic efficacy in a number of animal models. Furthermore, in clinical studies the favorable pharmacokinetics and bio-distribution of PEGylated liposomal doxorubicin have been shown to translate to significant activity against AIDS related Kaposi's sarcoma and against ovarian and breast cancers [6, 7]. Life enhancement products shows that conventional liposomes are good but PEGylated liposomes are better because two main advantages of PEGylated liposomes for delivering drugs or supplements are *increased bioavailability* and of *targeted delivery* to the organs or tissues that most need them [8]. K. Remaut were compared the intracellular distribution of non-PEGylated and PEGylated liposomes to check the endosomal degradation of the delivered phosphodiester oligonucleotides. The non-PEGylated liposomes efficiently escaped from the endosomes thereby releasing phosphodiester oligonucleotides (PO-ONs) in the cytoplasm of the cells. In contrast to non-PEGylated liposomes, PEGylated liposomes failed in protecting the PO-ONs they were carrying, leading to rapid degradation of the PO-ONs in the endosomal compartment [9].

A Carbon Nanotube is a tube-shaped material, made of carbon, having a diameter measuring on the nanometer scale. A nanometer is one-billionth of a meter, or about one ten-thousandth of the thickness of a human hair. As a group, Carbon Nanotubes typically have diameters ranging from <1 nm up to 50 nm. Their lengths are typically several microns, but recent advancements have made the nanotubes much longer, and measured in centimeters [10]. The widespread use of carbon nanotubes is severely limited by the difficult nature of processing and handling them in a facile manner because of its insolubility in a process-friendly solvent. One solution for this is the use of polymers, which not only solubilize nanotubes by encapsulation but also keep the intrinsic property of nanotubes intact [11]. Noncovalent functionalization of single-walled carbon nanotubes (SWCNTs) with phospholipid-polyethylene glycols (Pl-PEGs) was performed to improve the solubility of SWCNTs in aqueous solution. Evaluation of functionalized SWCNTs showed that the non-covalent functionalization protocol could considerably increase aqueous solubility, which is an essential criterion in the design of a carbon nanotube (CNT) -based drug delivery system and its biodistribution [12].

Treating cancer is a world wide issue. Highly toxic drugs are useful to prevent multiplication of cancer cells but they are too dangerous to deliver in a systemic manner. Now a day, Nanotechnology has begun to play a key role in detection and treatment of cancer. New research conducted at The School of Pharmacy has found that carbon nanotube-based delivery systems may have a significant impact in the fight against lung cancer [13]. The amount of loaded drug on a CNT is rather small. In this respect, liposomes (lipid vesicles) are employed for transporting a large amount of drug [14]. Liposomes are self-enclosed spherical vesicles composed of amphiphilic lipids. But they may limit the function because of bi-lipid layered membranes and they are rapidly cleared from blood plasma. In contrast, the tube shape of carbon nanotubes suggest that the chamber inside may be accessible to small molecules. The attributes of liposomes and nanotubes must be considered to determine their capability of encapsulating and transporting molecules [15]. The direct comparison between carbon nanotubes and liposomes demonstrates the potential advantages offered by carbon nanotubes for the intracellular delivery of therapeutic agents in vivo [16].

The aim of present work was to study the effect of PEGylation on liposomes and carbon nanotubes loaded with same drug methotrexate to compare the effectiveness of both nanotechnologies based formulations for targeting cancer cells.

MATERIALS AND METHODS

Materials

Multiwalled carbon nanotubes with 10-15 nm outer diameter, 2-6 nm inner diameter and 0.1-10 µm length were purchased from the Sigma-Aldrich, Germany. Soya phosphatidycholine was purchased from Life care innovation, Bombay. 1, 2 - Distearoyl-phosphatidylethanolamine-methoxy-polyethylene glycol conjugate-2000 (DSPE-mPEG 2000) was received as gift sample from the Sun Pharma Advanced Research Centre, Baroda, India. Methotrexate was received as a gift sample from the Zydus Cadila, Ahmedabad, India. All other chemicals were of laboratory grade purchased from local suppliers.

Methods

Method of preparation of PEGylated Liposomes [17]

TFH (Thin Film Hydration) method was selected for the preparation of Liposomes in this investigation due to non-tediousness and feasible at lab scale compared to other techniques (Figure 3).

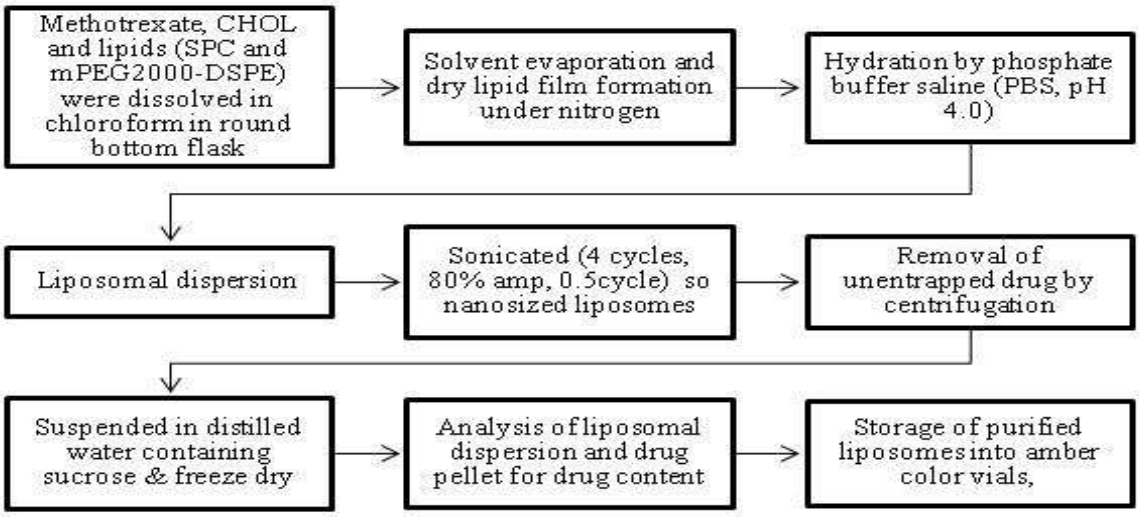


Figure 3: TFH method for preparation of liposmes.

PEGylated liposome were composed of SPC:CHOL: DSPE-mPEG 2000 in a 90:10:5 M% ratio. Briefly, methotrexate, CHOL and lipids (SPC and mPEG2000-DSPE) were dissolved in chloroform and dried in a rotary evaporator to form a thin film layer, which was re-suspended in phosphate buffer saline (PBS, pH 4.0) until completely hydrated. Then, the liposome dispersion was sonicated (4 cycle, 3min., 80% amp, 0.5cycle/sec.) in probe sonicator (RR-120, Ralsonics, Mumbai). Un-entrapped methotrexate was removed from the liposome suspensions by centrifugation and the liposome pellet was washed twice with PBS (pH 7.4). The pellet was then suspended in distilled water containing sucrose (molar ratio of sugar-to-lipid = 2.3), and freeze-dried. The final PEGylated liposome particles were stored in tight containers at 4 °C for further experiments. Table 1 contains specification of process parameters to prepare MTX Liposomes. Liposomal suspension was then characterized for vesicle size and percent drug entrapment (PDE).

Table 1: Specification for formulation of MTX Liposomes

Molar ratios	MTX : SPC		1:25
	SPC : CHOL		9:1
	Chloroform : methanol		2:1 v/v
Process parameters	Vacuum		600 mm Hg
	Solvent evaporation time		40 min
	Speed of rotation	Film formation	100 RPM (80 min)
		Hydration	80 RPM (75 min)
	Sonication		80% amplitude, 0.5 cycles/min, 4 cycles, 3 min.

Method of preparation of PEGylated Carbon Nanotubes [18]

MWCNTs (multiwalled carbon nanotubes) were functionalized by mixing MWCNTs: DSPE-mPEG 2000: MTX in a 1:8:4 ratios in water and sonicated in a bath sonicator (Figure 4).

First of all, MWCNTs: DSPE-mPEG 2000 (1:8) was dissolved in water by sonication for 90 min with 5 min time interval. Unbound surfactant was thoroughly removed by repeated filtration through 100 kDa filters (Millipore). The functionalized MWCNTs were then resuspended in phosphate-buffered saline (PBS, pH 7.4) by sonication in a bath sonicator and were mixed separately with the known concentration of methotrexate solution prepared in same buffered

saline. The mixture was kept overnight at 7.4 pH conditions. Suspension was used for further analysis of particle size and drug entrapment efficiency.

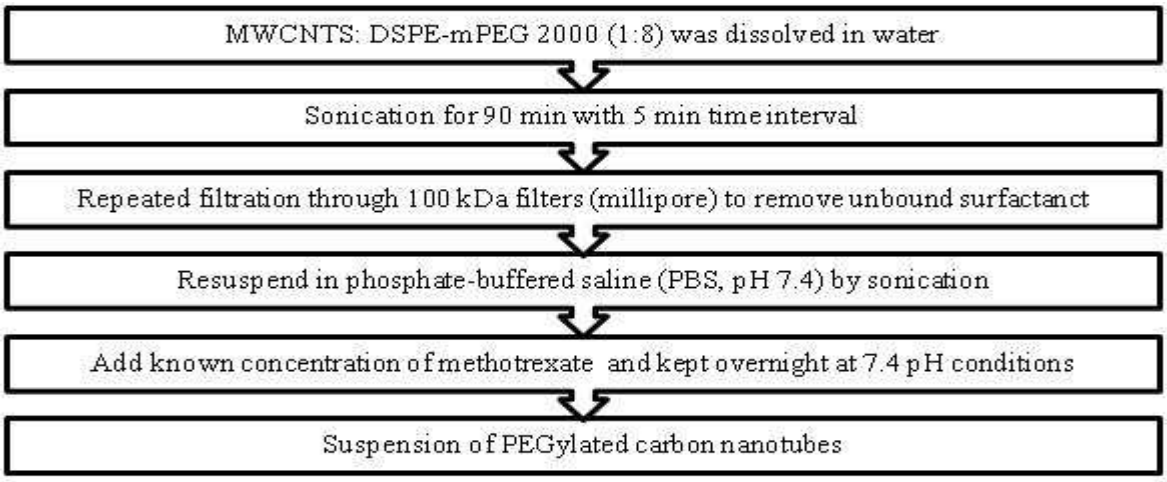


Figure 4: Preparation of PEGylated Carbon Nanotubes.

Characterization:

Drug entrapment efficiency [19]

The drug loading (drug incorporated onto the 1 mg functionalized MWNTs out of 4mg initially taken and on to liposomes) were determined by passing the 1ml formulation from Sephadex G-50 column (to remove un-entrapped drug), washing the column with 1 ml phosphate buffer pH 7.4 and collecting the 4 fractions of 0.5 ml. Dilute the fractions with phosphate buffer pH 7.4 and measure the absorbance at 259nm using phosphate buffer pH 7.4 as blank. Calculate the drug loading and % assay.

Particle size analysis

Particle size distribution study and zeta potential of the MWCNT conjugate & liposome conjugate was measured using Zetasizer Nano ZS (Malvern instruments, U.K.).

In-vitro drug release study [20]

The *in vitro* release study of Methotrexate from the MWCNTs formulation and liposomal formulation was determined using dialysis membrane. Briefly, 1 ml of MWCNTs formulation/liposomal suspension was taken in a dialysis tube (Mol. Wt. cut-off 12 000; HIMEDIA, Mumbai, India Himedia) and was suspended in phosphate buffer at a specified pH. Drug release from the formulations was determined by estimating drug content in the samples withdrawn at convenient intervals of time for 48 hours.

Stability study [21]

Stability study of both PEGylated liposomes and PEGylated MWNTs formulations were carried out at room temperature (R.T.) and at refrigerated conditions (Freeze) for 1 month. Drug assay (%) and particle size were determined for samples withdrawn at specified time interval.

RESULTS

Functionalization and drug loading of carbon nanotubes [18]

Multiwalled carbon nanotubes were successfully functionalized by DSPE-mPEG, which makes them well dispersible in distilled water. The drug loading efficiency of carbon nanotubes were found to be 56.5% with 185.1 nm particle size. One can load about 2.26 gm of methotrexate on 1

gm of carbon nanotubes. Table 2 shows optimized formula to prepare well functionalized drug loaded carbon nanotubes.

Table 2: Formula of PEGylated carbon nanotubes.

Ingredients	Quantity
Mutiwalled carbon nanotubes	1 mg
DSPE-mPEG 2000	8 mg
Methotrexate	4 mg
Phosphate buffer pH 7.4	5 ml
Sonication time	90 min with 5 min interval
Incubation period for drug loading	24 hours

Particle size analysis and zeta potential measurement of MWNT conjugate [18]

The PEGylated carbon nanotubes showed a mean particle size of 189.7 nm with 0.215 PDI and 100% peak intensity when the formulation was reconstituted using pyrogen free water (Figure 5). The Zeta potential of the same formulation was found to be -25.8 mV (Figure 6).

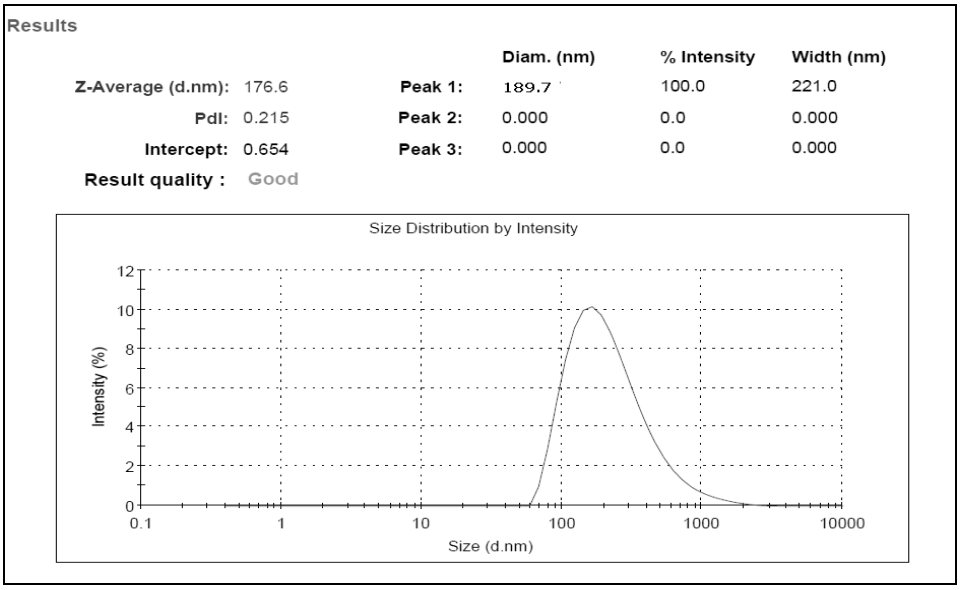


Figure 5: Particle size of MWNTs formulation

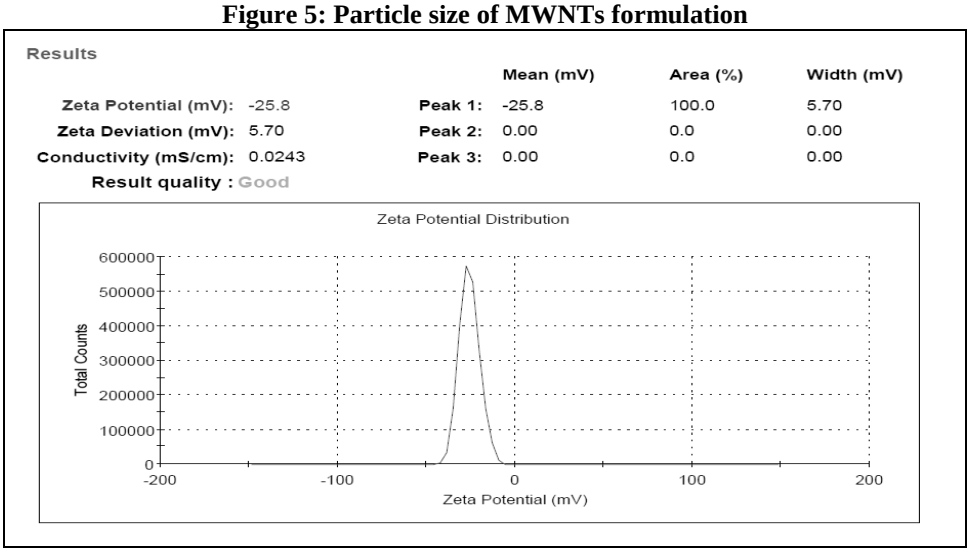


Figure 6: Zeta potential of MWNTs formulation

Characterization of liposomes

Characterization parameters of MTX Liposomes are shown in Table 3. Figure 7 and 8 represents particle size and zeta potential of MTX Liposomes respectively.

Table 3: Characterization parameters of MTX Liposomes

Characterization parameters	Results
% Drug entrapment	67.93% ± 0.278
Particle size (PDI)	80.7nm (0.239)
Zeta potential	-19.4

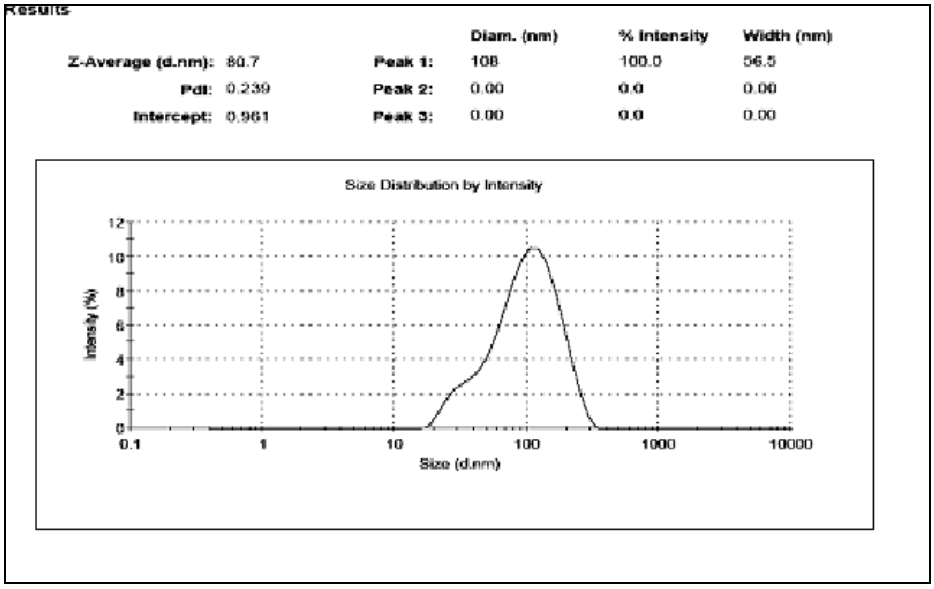


Figure 7: Liposomal size after Sonication.

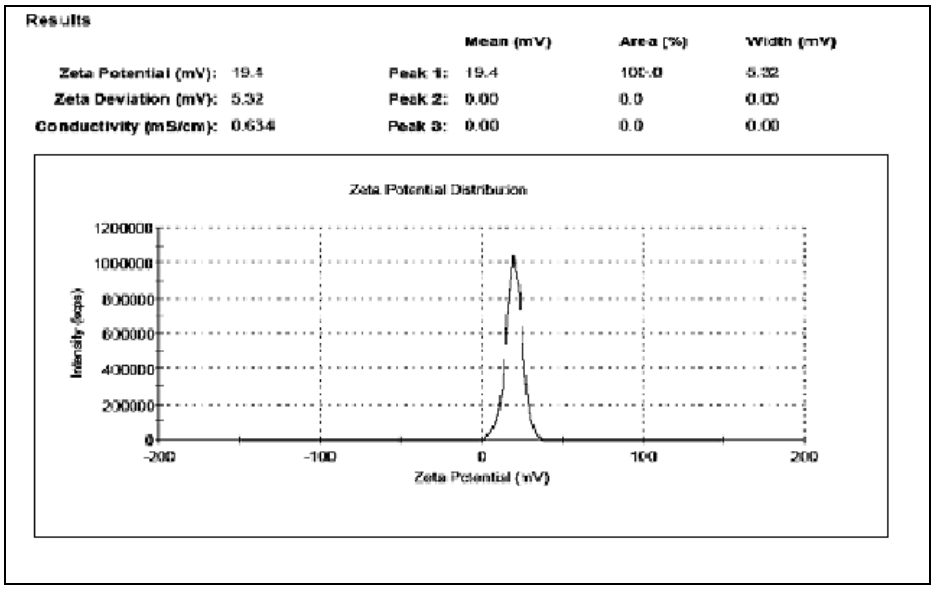


Figure 8: Zeta potential of lyophilized liposomes.

In-vitro release study

Figure 9 shows the In-vitro drug release profile of both PEGylated formulation MWNTs-MTX and Liposomal MTX in phosphate buffer pH 7.4 & 5.8 against plain drug suspension.

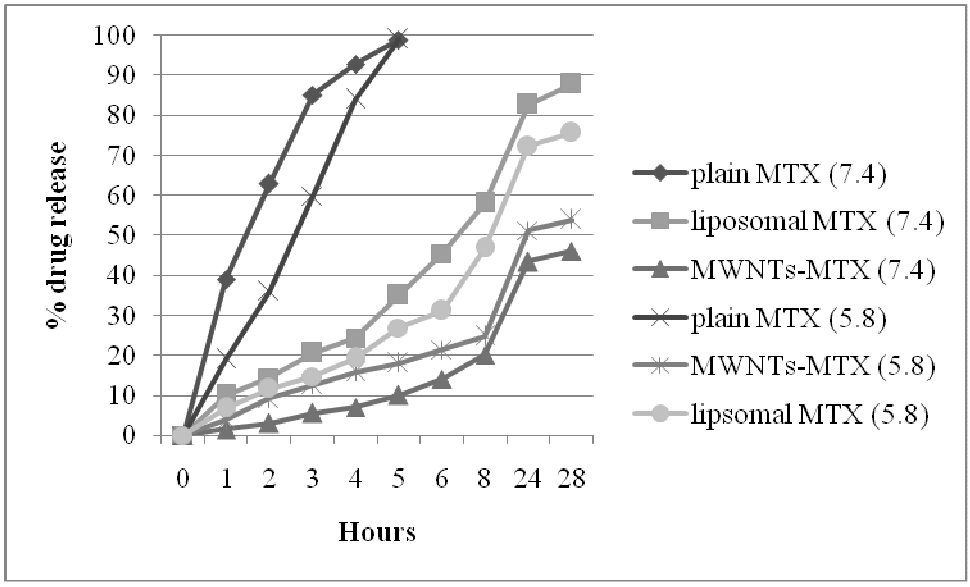


Figure 9: In-vitro release study profile.

The percentage release behavior of formulation MWNTs-MTX in acidic environment i.e. in pH 5.8 was greater (53.84% after 28 hours) as compared to pH 7.4 (46.08% after 28 hours). In contrast, liposomes show less drug release in acidic environment (75.7%) than at pH 7.4 (87.86%) after 28 hours.

Stability study

Figure 10 and 11 represents stability studies of MWNTs and LIPOSOMES at room temperature (RT) and refrigerated conditions (Freeze). Sampling was done after every 2 weeks and then % drug retained and particles size of formulations were measured out. Figure 10 shows Percentage drug retained in both formulations and figure 11 explains increased particles size during storage at different storage conditions.

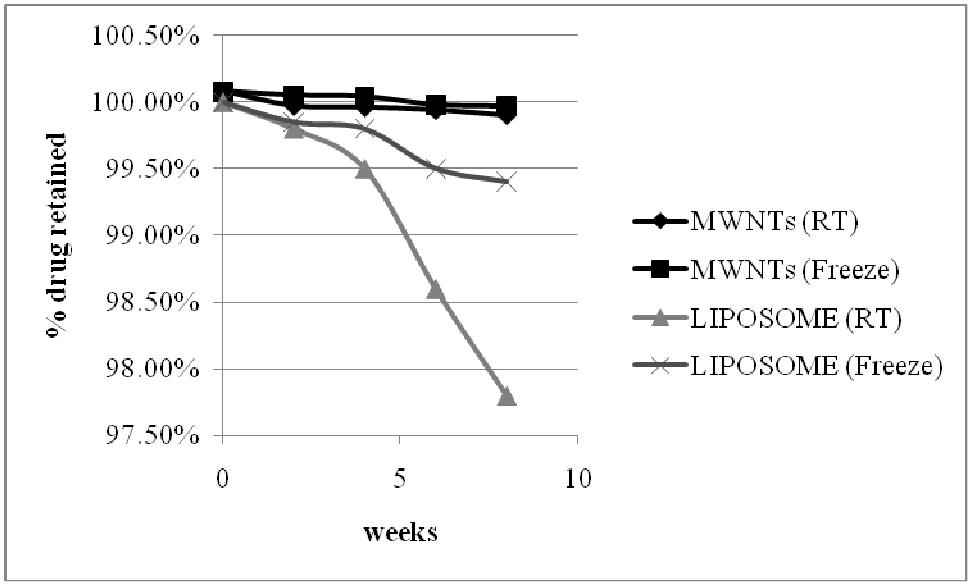


Figure 10: Percentile drug retained during stability study

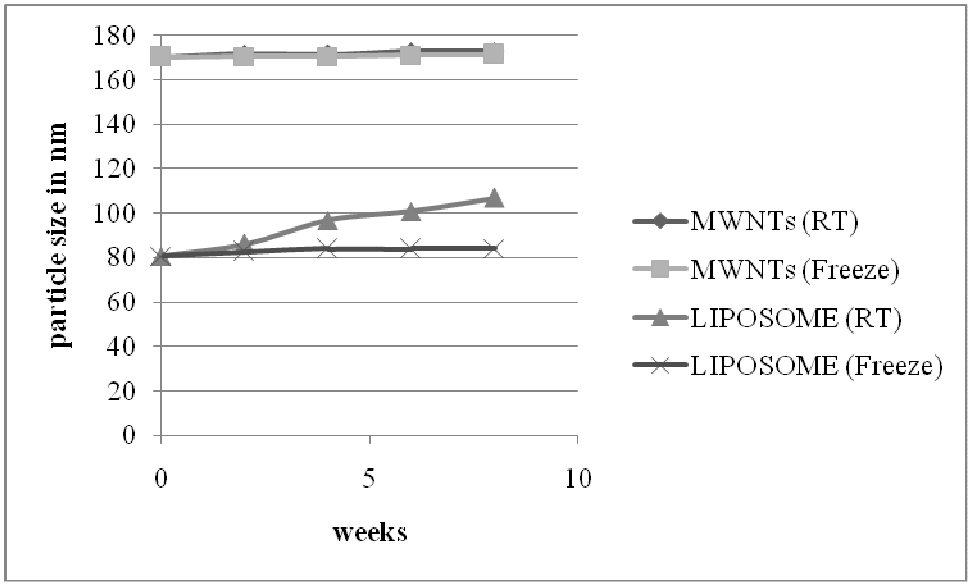


Figure 11: Particle size (in nm) during stability study

DISCUSSION

Carbon nanotubes, wrapped cylinders of graphitic carbon, are ultimately strong nanofibers with a Young's modulus of ~1 TPa and tensile strength of ~100 GPa. Such amazing "dream" structural material, nearly 100 times stronger than standard steels, must and will be utilized for individual or composite applications in the 21st century [22].

Structurally, Liposomes have semi fluidic nature which is much less rigid than sp^2 carbon network of nanotube. This also provides difficulties when considering how CNT might be eliminated from biological systems, by either breaking down or physically disposing of them. Not the least of these includes the current inability to select nanotubes according to their diameter, size or chirality. Another experimental drawback is cutting of nanotubes, so that they are small enough to be functionalized and made soluble in aqueous solutions. They also must be long enough to hold the internalized molecules without losing all of their contents before reaching the target. Finally, a nanotube containing drug molecules must be emptied once it reaches its destination [23, 24].

Competing delivery systems for molecules into the body do exist, such as polymer nanoparticles or capsules known as Liposomes, which are essentially made of artificial cell membranes. One advantage carbon nanotubes have over these competitors "is one can really engineer carbon nanotubes and nanoparticles very precisely, almost atom by atom, all essentially identical, which is much more difficult with Liposomes or polymer systems [25, 26].

The modification of therapeutic molecules through the attachment of poly (ethylene glycol) [PEG] moieties ('PEGylation') are the most common approaches for enhancing the delivery of parenteral agents. PEGylation reduces renal clearance and, for some products, results in a more sustained absorption after subcutaneous administration as well as restricted distribution. These pharmacokinetic changes may result in more constant and sustained plasma concentrations, which can lead to increases in clinical effectiveness when the desired effects are concentration-dependent. Maintaining drug concentrations at or near a target concentration for an extended period of time is often clinically advantageous, and is particularly useful in therapy [3]. Simple modification with polyethylene glycol (PEG) is not only capable of improving the

pharmacological properties of a drug, especially for peptide and protein therapeutics, but has also to be considered with regard to its life cycle extension [27]. In-vitro profile shows that plain drug was rapidly released from suspension within 5 hours up to 98%. Whereas, both PEGyalted formulations shows long time steady drug release (Figure 9).

As far as concern with stability study, one can predict that drug was retained in MWNTs even after 8 weeks because of their length, whereas decreased drug retention was seen with liposomes (Figure 10). PEGylated MWNTs conjugate maintain their particle size during storage, but the particle size of Liposomal conjugate was constantly increases with time (Figure 11).

PEG quality is important in order to achieve reproducible PEGylation and reliably meet the specification of the PEGylated drug [27]. PEG is obtained by chemical synthesis and, like all synthetic polymers; it is poly-disperse, which means that the polymer's batch is composed of molecules having different number of monomers, yielding a Gaussian distribution of the molecular weights. Now a days, because of the development of synthetic and purification procedures, PEGs on the market are less poly-disperse than those employed initially, but the poly-dispersivity problem must be still taken into consideration, especially when dealing with low molecular weight drugs, either peptide or non-peptide drugs, where the mass of linked PEG is more relevant for conveying the conjugate's characteristics, mainly those related to the molecular size. The high water coordination of the polymer increases the PEG's hydrodynamic volume up to 3–5 times that of a globular protein having the same molecular weight, thus decreasing the polymer kidney clearance threshold and the linear and flexible structure of PEG chains that help the polymer to cross the glomerular membranes by a 'snake-like' movement. In this study, DSPE-mPEG was used for PEGylation, is widely used lipid conjugated surfactant used for PEGylation of various nanotechnology formulations like liposomes, nanoparticles etc [28, 29].

It was fact that direct comparison of carbon nanotubes and Liposomes is not easy because they are much similar in many ways like hydrophobicity, size restriction, storage and delivery mechanics, among other properties [30]. Table 4 shows difference between carbon nanotubes and liposomes.

Table 4: Difference between carbon nanotubes and Liposomes

Carbon nanotubes	**Liposomes**
Not spherical. Long tubes.	Spherical and colloidal.
Easy to engineer and drug load on to nanotubes.	Very tedious method to make.
More drug pay load because of large surface area.	Less drug incorporation as compared to carbon nanotubes.
No uptake by reticuloendothelial system (RES), so long circulation in body.	Extensive uptake by tissues of RES, so rapidly eliminated from body.
Toxic to body in some way.	Non toxic to body.
All types of drugs can be incorporated which have $-NH_2$ or –COOH functional group.	Only lipid soluble drugs can be incorporated in hydrophobic region of Liposomes.

Following table 5 shows some comparative data of MWNTs-MTX and Liposomal MTX. The results indicate that % drug entrapment and Particle size of Liposomal MTX is much good as compared to nanotube formulation. But drug: lipid ratio is higher in Liposomes (1:25) (Table 1) than the carbon nanotubes (1:8) (Table 2). Higher the ingredients and its quantity higher will be the complications [31].

Table 5: comparison data of MWNTs-MTX and Liposomal MTX.

Characterization parameters	Liposomal MTX	MWNTs-MTX
% Drug entrapment	67.93% ± 0.278	56.5% ± 0.184
Particle size (PDI)	80.7nm (0.239)	185.2nm (0.273)
Zeta potential	-19.4	-25.8

Secondly, Liposomes have extensive uptake by tissues of reticuloendothelial system. So PEGylation makes them render block from renal clearance. In-vitro study represents that MTX from Liposomal formulation was released up to 88% after 28 hours at pH 7.4, but it was lesser at pH 5.8. The reason is that unlike carbon nanotubes, they can not release the drug at acidic environment. But from the PEGylated nanotubes, the release was slow and steady at pH 7.4, whereas fast release of drug from carbon nanotubes was seen at pH 5.8 (Figure 9).

Additionally, carbon nanotubes can easily eliminated from the body after they are reaching at destination [32]. So they are not much toxic to body. So carbon nanotubes are able to deliver the drug to a cancer cell and avoid other normal cells [33].

Reporting its work in the journal ACS Nano, a research team led by Hongjie Dai, Ph.D., an investigator in the Center for Cancer Nanotechnology Excellence Focused on Therapy Response, showed that polymer-coated single-walled carbon nanotubes spontaneously absorbed the cancer drug doxorubicin onto their surfaces when the drug was added to the nanotubes dissolved in water. The resulting construct contained approximately 50 to 60 percent doxorubicin by weight, far higher than the 8 to 10 percent obtained with either liposomes or dendrimers. Carbon nanotubes retained their drug payload when dissolved in normal physiological buffer and blood serum, but the drug is released quickly from the nanotubes in the acidic environment (characteristic of the intracellular domain of tumor cells) [34].

Summarizing study of PEGlation approach has the most relevant advantages are the prolonged body-residence time, which allows less frequent administrations and the increase in stability towards renal clearance. These advantages of PEGylation allowed this technique to create blockbuster products, such as PEGylated MWNTs-MTX and LIPOSOME MTX. In particular, the increasing use of PEGylation was possible because of the availability of PEGs with different molecular weights and activation forms needed to respond to the various drug-modification requirements.

CONCLUSION

From the results and discussion, it is concluded that formulation of MWNTs was better in much ways as compared to Liposomal MTX. PEGylation of MWNTs gives targeted action on cancer cell environment (pH 5.8). Effect of PEGylation shows influence results in case of carbon nanotubes than liposomes. Particle size distribution and drug loading efficiency of liposomes as well as carbon nanotubes were effective to be given by IV route. In-vitro drug release profile of MWNTs-MTX shows a greater drug release of MTX in acidic environment. Stability study also proves that the drug retention is better in MWNTs-MTX conjugate than liposomes. Finally, effect of PEGylation shows better results in case of carbon nanotubes than liposomes.

Acknowledgement
I make my regards to my guide Dr. R.S.R.Murthy and M. S. University of Baroda, Vadodara for providing best lab facilities necessary for completion of research project. I am very much thakful

to SPARC, Vadodara and Zydus Cadila, Ahmdedabd for providing gift sample of DSPE-mPEG , SPC, Cholesterol and Methotrexate respectively.

REFERENCES

[1] PEG reagents, Thermo scientific, pierce protein research products, © **2011** Thermo Fisher Scientific Inc., 3747 N Meridian Rd, Rockford, IL USA 61101.
[2] PEGylation, Wikipedia, the Free Encyclopedia.
[3] Francesco M.Veronese and Gianfranco Pasut, *Drug Discovery Today*, **2005** Nov: 10(21):1451-58.
[4] Harris JM, Chess RB, *Nat Rev Drug Discov*. **2003** Mar; 2(3):214-21.
[5] Frank Leenders and Ralf Kraehmer, PEGylation technology and PEGylated drugs, http://www.celares.de. Viewed online on 30th July, **2011**.
[6] Prajapati BG, A Review on PEGylated Liposome in Cancer Therapy and in Delivery of Biomaterial, pharmainfo.net, Tue, 11/13/**2007**.
[7] Ichikawa K, Hikita T, Maeda N, Takeuchi Y, Namba Y, Oku N, *Biol Pharm Bull*. **2004** Mar; 27(3):443-4.
[8] PEGylated Liposomes Increase Bioavailability, www.life-enhancement.com.
[9] K. Remaut, B. Lucas, K. Braeckmans, J. Demeester, S.C. De Smedt, *Journal of Controlled Release*, **2007**, 117(2); 256-266.
[10] Carbon nanotubes, www.nanocyl.com/CNT-Expertise-Centre/Carbon-Nanotubes.
[11] O.-K. Kim, J.Je, J.W. Baldwin, S. Kooi, P.E. Pehrsson, and L.J. Buckley, Water-Soluble Carbon Nanotubes, www.nrl.navy.mil.
[12] Hadidi N, Kobarfard F, Nafissi-Varcheh N, Aboofazeli R, *International Journal of Nanomedicine*, **2011**, 2011(6);737 - 746
[13] Can Carbon Nanotubes Help 'Silence' Lung Cancer? By Materials Views Staff published: **2009**-04-23.
[14] Faina Karchemsky and Oren Regev, Drug-transportation by carbon nanotubes, www.bgu.ac.il.
[15] Austin Kirschner, Stephen Wilson, David Santosa, Liposomes vs. Carbon Nanotubes as Small Molecule vessels for drug delivery. www.electrochem.org.
[16] Podesta JE, Al-Jamal KT, Herrero MA, Tian B, Ali-Boucetta H, Hegde V, Bianco A, Prato M, Kostarelos K. Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model. Small. **2009** May;5(10):1176-85
[17] Tao Yang, Min-Koo Choi, Fu-De Cui, Jung Sun Kim, Suk-Jae Chung, Chang-Koo Shim, Dae-Duk Kim, *Journal of Controlled Release* 120 (**2007**) 169 – 177.
[18] Mrs. Chetana D. Modi, Mr. Suketu J. Patel, Mr. Alpesh B. Desai, Dr. R. S. R. Murthy, *Journal of Applied Pharmaceutical Science*, **2011**, 1(5), 103-108.
[19] María Castro-Puyana, Ivonne Lammers, Joost Buijs, Cees Gooijer and Freek Ariese, *Analytical and Bioanalytical Chemistry*, 10.1007/s00216-011-4991-6.
[20] H.K. Kimelberg, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1976**, 448(4);531-550.
[21] Stability testing of active substances and pharmaceutical products, World Health Organization, Working document QAS/06.179.
[22] Dmitri Golberg, Surface Functionalization of Nanotube Fillers: A Review, Nanotechnology Now, published: 2011-07-26.
[23] Kostas Kostarelos, Nano World: Making safer carbon nanotubes, Physorg.com, science:phsics:tech:nano:news.
[24] Anna Lewcock, Carbon nanoneedles for Drug Delivery, 08 February **2007**.

[25] Maurizio prato,Kostas kostarelos, Functionalized carbon Nanotubes in Drug Design and Discovery, Acc. Chem. Res., **2008**, volume 41, issue 1, page no. 60-68.
[26] Michael Berger, Carbon nanotubes as effective cancer killers, Copyright 2007, Nanowerk LLC, Posted: July 11, **2007**.
[27] Dr Frank Leenders, PEGylation technology and biopharmaceuticals, Biopharmaceuticals, June **2006**, 39-40.
[28] Rathore SS, Ghosh PC., *Int J Pharm*. **2008** Feb 28; 350(1-2):79-94. Epub 2007 Aug 26.
[29] Neeraj Vij, Taehong Min, Rhul Marasigan, Christopher N Belcher, Steven Mazur, Hong Ding, Ken-Tye Yong, and Indrajit Roy, *J Nanobiotechnology*. **2010**; 8: 22.
[30] Sofou S, Surface-active liposomes for targeted cancer therapy, Nanomedicine (Lond). **2007** Oct; 2(5):711-24.
[31] Choosing ingredients, http://www.unilever.com/sustainability.
[32] Nanomaterials, public health, DG health and consumer protection, EUROPA.
[33] Dai, H. *Surf. Sci.* **2002**, *500*, 218–241.
[34] Zhuang Liu, Xiaoming Sun, Nozomi Nakayama-Ratchford, and Hongjie Dai, *ACS Nano*, **2007**, 1(1), 50–56,.