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Raising the Physical and Chemical Properties and Performance of Nanovesicles Derived from Red Blood Cells[1]

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ABSTRACT

Physiological obstacles reduce medication efficacy for many promising compounds. **T**he main aim of the study is to raise the Physical and Chemical Properties and Performance of Nanovesicles Derived from Red Blood Cells**.** Statistical information was shown using mean and standard deviation for all variables (SD). All statistical tests were performed in Origin Pro8.5. Free CPT was shown to be very toxic, causing cardiac cachexia due to cancer treatment, intestinal damage, and hepato-renal failure.

Keywords: *Physiological; Performance; Nanovesicles; Degeneration; Toxicity*

INTRODUCTION

Physiological obstacles in medication distribution reduce the efficacy of many promising therapeutic candidates. Cancer is a well-known example since it is so difficult to get precise medicine delivery to malignant tissue. Using nanoparticles as a medicine delivery mechanism may help alleviate this issue to some degree. In comparison to free medication formulations, those based on nanoparticles (NPs) have the potential for increased therapeutic effectiveness and decreased adverse effects. Most chemotherapy medicines have molecular weights that are below the renal cutoff range and are therefore eliminated from the body with relative ease. As a consequence, tumour cells have a limited supply of medications to combat the disease. Nanoparticles, on the other hand, may be engineered to encapsulate medications, and their optimum size range (50-200 nm) precludes fast renal clearance, thereby enhancing pharmacokinetics. Some of the most effective cytotoxic agents are poorly water-soluble, necessitating either the use of other solvents or chemical modifications to the drug itself. As a result of the drug's chemical alteration and the added toxicity of the solvents, its efficacy may decrease. In this context, nanoparticles may load medications that aren't water soluble with a high yield and protect them from premature destruction. Multi-drug resistance is another issue that might reduce the efficacy of chemotherapy. As time goes on, cancer cells may become resistant to taking in active drug molecules; in this situation, drug-loaded nanoparticles might provide an alternate entryway into the cell via endocytosis. Solid tumours are characterised by aberrant vasculature. Blood arteries inside tumours sometimes have abnormalities in their endothelium, making it easier for macromolecules, such as nanoparticles, to enter the interstitial space within the tumour. Hence, medications may be directed specifically to the tumour, reducing their impact on healthy tissues. Moreover, the possibility of medications losing their bioactivity makes the scope of chemical alteration of the pharmaceuticals for active targeting much less. Using nanoparticles (NPs) as a delivery vehicle is a simple solution to this issue since only the NPs' surface can be modified with actively targeted ligand without compromising the drug molecules' integrity. Finally, nanoparticles' adaptable physicochemical features allow for site-specific cargo delivery, and their capacity to load pharmaceuticals with different chemical structures increases their solubility and bioavailability.

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LITERATURE REVIEW

Elkanzi, Nadia & Hrichi, Hajer & Alolayan (2022) Because of their ketoethylenic moiety (CO-CH=CH-), species deriving from chalcones are highly prized. Because of advances in heterocyclic chemistry, chalcone derivatives may now be synthesised, which have been the subject of biological investigation as potential treatments for certain diseases. An analysis of the current scientific literature suggests that numerous chalcone-heterocycle hybrids show potential as new therapeutic candidates, with activities that are either equivalent to or superior to the gold standards. As a result, this analysis has the potential to aid in the creation of novel, highly effective therapeutic medications that build on established methods.

Khasawneh, Mohammad & AlKaabi (2022) Several new 8-hydroxyquinoline urea and thiourea compounds have been developed, synthesised, and tested for their anticancer properties. Novel chemical structures were identified by use of spectroscopic methods including 1H NMR, 13C NMR, and mass spectrometry. The MTT test was used to evaluate the cytotoxicity towards MCF7 and MDA-MB-231 cell lines in vitro. The cytotoxicity of six of the eleven compounds synthesised was shown, with IC50 values ranging from 0.5 to 42.4 M. Compounds 5b, 5c, 5f, and 6b-d were all included. Using DAPI and ethidium bromide/acridine orange labelling with fluorescence microscopy, we observed apoptotic characteristics in MCF-7 cells treated with 5b chemical. Hydrogen bonds, -contacts, and hydrophobic interactions were found between these compounds and the active site of poly (ADP-ribose) polymerase-1 (PARP1), B-cell lymphoma-extra large (Bcl-xL), and PARP5A, three of the sixteen possible breast cancer proteins (Tankyrase 1). It was found that the docked postures of these molecules at the active sites of these targets were quite similar.

Gujjarappa, Raghuram & Kabi (2022) In medical chemistry, natural products, and synthetic chemicals, imidazole and its derivatives are among the most significant and commonly utilised heterocycles. Imidazole derivatives may demonstrate a broad variety of biological and pharmacological effects because to the imidazole ring's distinctive structural properties. These derivatives can bind to various enzymes and receptors via a number of weak interactions. Many imidazole compounds have seen extensive clinical application in treating a broad variety of disorders, indicating that they have tremendous untapped potential. This chapter summarises contemporary studies in the area of medicinal chemistry examining the effectiveness of Therapeutic applications of imidazole derivatives: anti-inflammatory, anti-cancer, anti-viral, and antibacterial.

Oluwakemi, Ebenezer & Shapi (2022) To put it simply, pyrazoles are nitrogen-containing heterocyclic compounds with five members. As a result of their significance in the pharmaceutical industry, these molecules have received a lot of study. Meanwhile, pyrazole derivatives have been created as target compounds and shown to exhibit a variety of biological actions, including antituberculosis, antibacterial, antifungal, and anti-inflammatory effects. In this article, we will examine the literature and describe the findings from studies that have been conducted on the synthesis and biological activity of pyrazole derivatives. Scopus was queried for all articles published on the synthesis and biological activity of pyrazole derivatives between January 2018 and December 2021.

Miao, Yu-hang & Hu, Yu-heng & Jie (2019) The benzofuran compounds are a naturally occurring family of chemicals. Anti-tumor, anti-bacterial, anti-oxidative, and anti-viral actions are only some of the many examples of powerful biological activity found in most benzofuran compounds. Because of their broad range of possible uses, benzofuran compounds are gaining interest from scientists in the chemical and pharmaceutical industries as promising natural medicinal lead molecules. Examples include the discovery and utilisation of new benzothiophene and benzofuran scaffold compounds as anticancer medicines, and developing a new macrocyclic benzofuran compound with anti-hepatitis C viral activity that shows promise as a therapy for hepatitis C disease. In recent years, new strategies for building benzofuran rings have been identified. A unique free radical cyclization cascade is employed to produce a tough benzofuran derivative, considerably simplifying the synthesis of a series of difficult polycyclic benzofuran molecules. Proton quantum tunnelling allows the production of a benzofuran ring with a high yield and minimal side reactions, making it a promising method for building larger benzofuran ring systems. This review compiles the most up-to-date research on benzofuran derivatives, including topics such as their chemical production, biological activity, therapeutic potential, and major natural product sources.

METHODOLOGY

Materials include foetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), antibiotic-antimycotic, 4',6-diamidino-2 phenylindole (DAPI), trypsin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-

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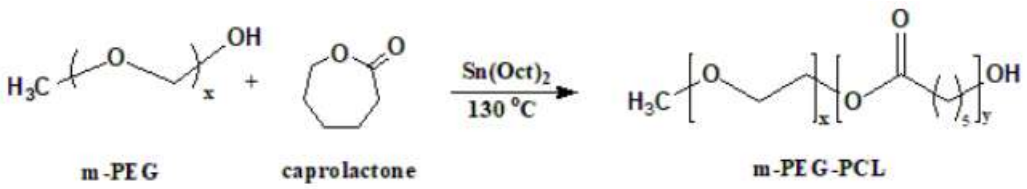
diphenyltetrazolium bromide were purchased from Invitrogen (MTT). We got our hands on a Spectrum Laboratories tiny float-a-lyzer with an 8000 MWCO capacity. If you need A549 cell lines, you may acquire them from the National Center for Cell Sciences (NCCS) in Pune, India. Sigma was contacted for the acquisition of methoxy PEG 5kDa (mPEG), caprolactone, CDCl3, stannous octoate, and toluene. Merck supplied us with camptothecin and acetonitrile. Aurogreen, which included ICG in the form of its sodium salt, was a commercially available product.

RESULTS

Characterization of di-block PEG-PCL polymer

It was possible to determine the structure, chain length, and molecular weight distribution of PEG-PCL diblock co-polymer (Figure 4.1). Nanoparticle size distribution and other features may not be repeatable if the polydispersity of the produced polymer is significant. Hence, 1H NMR, FTIR, DSC, and GPC were used to completely characterise the produced polymer. PEG (at about = 3.5 ppm) and PCL (at around = 3.9 ppm) blocks can be identified in the 1H NMR spectra (Figure 4.1a). Like the -OC2H5 of PEG, the -CH2 next to the CO group of PCL, and the -CO group of PCL, distinctive 13C peaks were found at δ= 72 ppm, 36.8 ppm, and 175 ppm, respectively (Figure 4.1b). Our results corroborated the synthesis of PEG-PCL copolymer and were in agreement with those described in the literature. In addition, 1H NMR was used to calculate the molecular weight distribution (Mn). The PCL block's chain length was calculated to be 9.1 kDa, whereas the Mn chain length was calculated to be 14.1 kDa. This results in a copolymer with the molecular formula PEG5kDa:PCL9.1KDa. Gel permeation chromatography revealed a molecular weight distribution with a polydispersity index (D) of 1.06 and a weighted average molecular weight (Mw) of 13.9. (Figure 4.1c, Table 4.1). The copolymer's low polydispersity index made it an excellent material for creating micelles with consistent, repeatable physiochemical properties. Peaks indicative of C=O at 1722 cm-1 and two C-H stretches, one for each block, were seen in the FTIR spectrum of the polymer (Figure 4.1d). In order to clear the samples of any previous thermal history before DSC analysis (Figure 4.1e), They were heated to much over their melting point, cooled to crystallise at a rate of 10°C/min (cycle 2), and then heated again at the same rate (cycle 3).



Scheme 4.1: Ring-opening polymerization reaction-catalyzed chemical synthesis of mPEG-PCL

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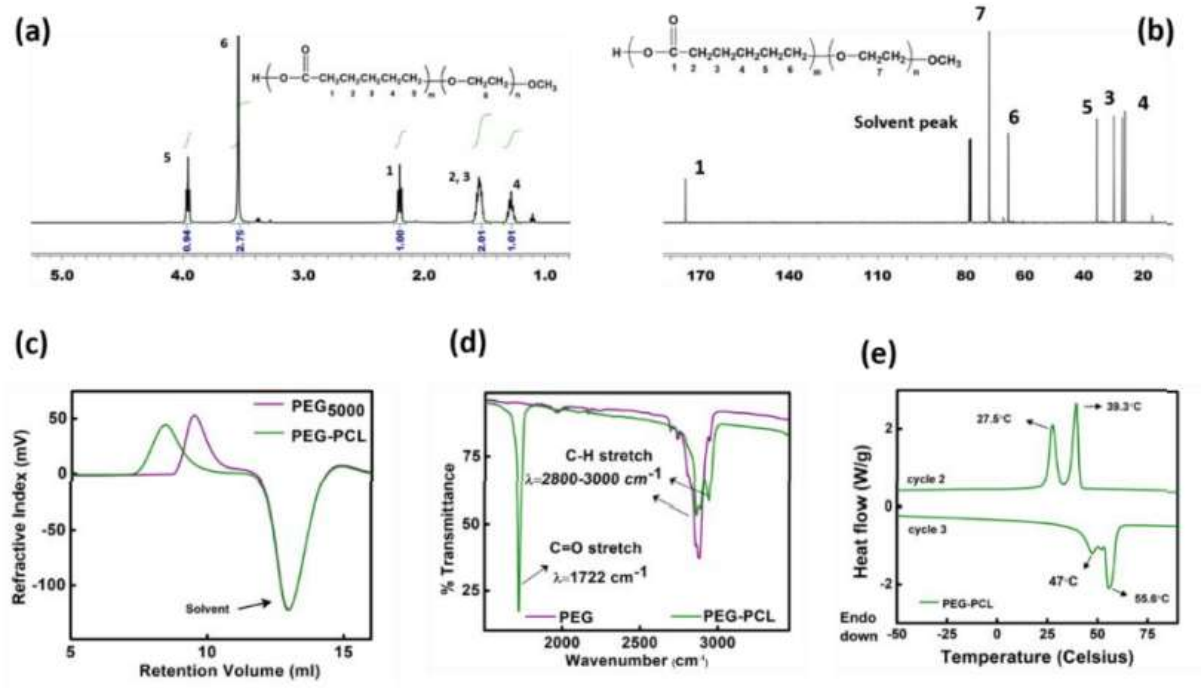


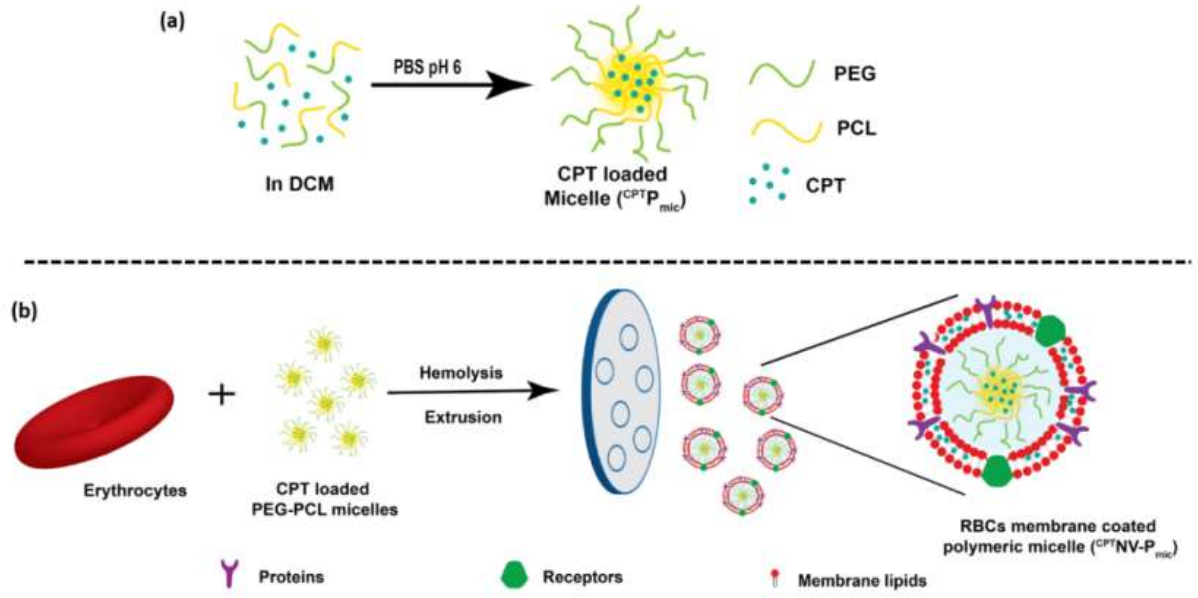
Figure 4.1: Chemical characterization of poly(ethylene glycol)-poly(ethylene chloride) (PEG-PCL)

Table 4.1: GPC analysis of the polymers

Sample ID	Replicates	M_w (g/mol)	M_n (g/mol)	Đ	Rh (nm)	IV (dL/g)	Recovery (%)
PEG-5000	i	5,218	4,459	1.17	2.938	0.4063	94.6
	ii	5,378	4,610	1.16	2.815	0.4121	93.8
	Average	**5,298**	**4,534**	**1.165**	**2.876**	**0.4092**	**94.2**
PEG_PCl	i	13,687	13,039	1.05	5.460	0.7586	90.1
	ii	14,121	13,075	1.08	5.661	0.8102	88.5
	Average	**13,904**	**13,057**	**1.06**	**5.560**	**0.7840**	**89.3**

Polymeric micelles and red blood cell membrane-coated micelles: characterization

Micelles were made by evaporating the solvent (Scheme 4.1a). The DCM was added gradually to the PBS while the polymer and medication were being dissolved. During the heating process, the solvent evaporates, and the medication precipitates inside the micelles. They were somewhat spherical in shape and measured between 50 and 80 nm in size (Figure 4.2a). DLS measurements showed micelle sizes to be somewhat larger than those obtained by TEM (90-120 nm), with PDI values between 0.01-0.2. These results imply that the micelles are both reproducible in size and monodisperse. As mentioned before, this is because the PEG-PCL polymer chains have a low polydispersity (Table 4.1).



Scheme 4.2: (a) Amphiphilic PEG-PCL micelles were loaded with CPT and then encapsulated in erythrocyte membrane-derived nanovesicles.

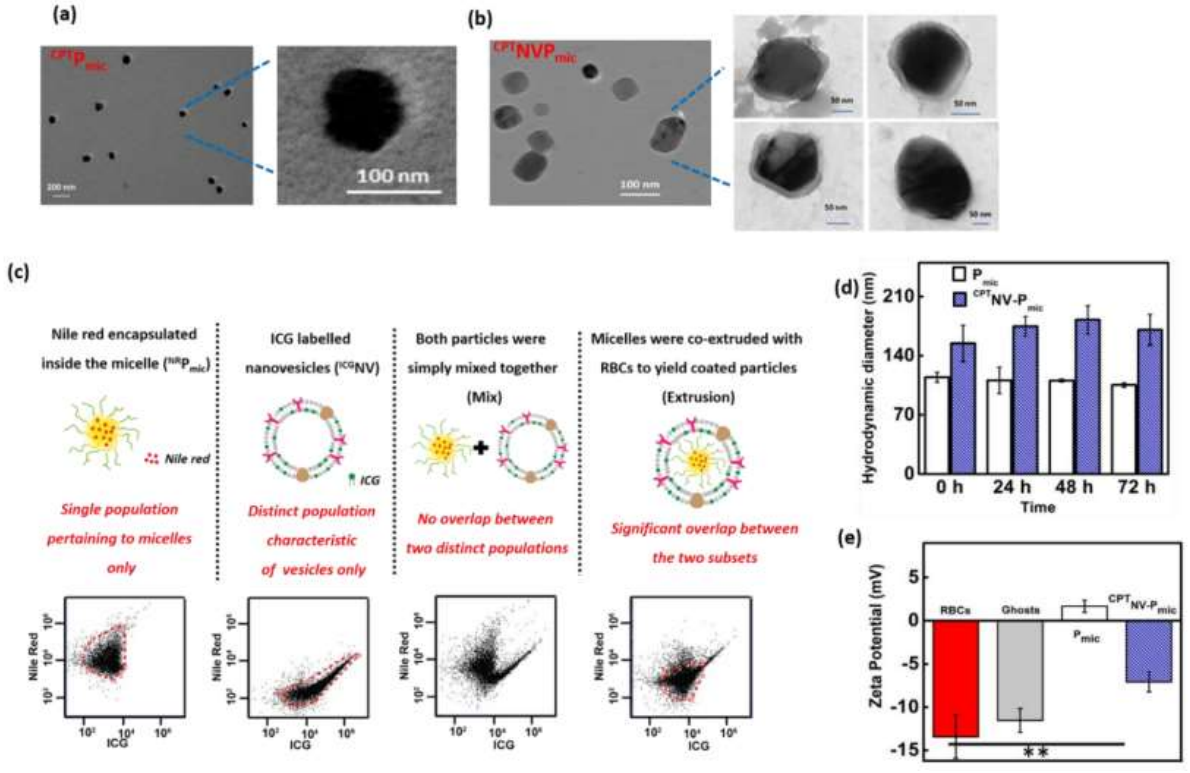


Figure 4.2: Properties of polymeric micelles and RBC membrane-coated micelles are being investigated.

In-vitro physio-chemical characteristics of CPTNV-Pmic

We generated CPT-loaded nanovesicles, with the lipophilic drug mostly sequestered inside the vesicle membrane as per a previously published method. In the introduction, we discussed some of the issues with the proposed method for delivering the hydrophobic medicine directly into lipid bilayers. To get around these disadvantages, we envisioned a hybrid (polymeric-natural vesicle) system that would not only provide the stealth

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coating of RBCs but also protect the drug in its natural state by enhancing the interaction of the drug with a polymeric micelle in the core of the RBC vesicles that were prepared. After loading the drug into the polymer at mass ratios (w/w) ranging from 1:1 to 10:1, the encapsulation efficiency was determined using centrifugation. To do this, we measured the CPT concentration of suspended micelles and used centrifugation at 1000 g to selectively precipitate the unencapsulated medication. A 1:1 PEG-PCL: CPT ratio only managed to encapsulate 15% of the drug. 4.3a, Figure The precipitation of CPT in DCM during dropwise addition of the solvent to the aqueous phase may account for the low encapsulation efficiency. Due to the aggregation of CPT and the polymeric micelles at polymer micelle sites, the resultant PBS solution included micron-sized particles with a PDI> 0.9: drug ratios of 1:1 and 2:1. (Figure 3.3a). The loading efficiency of the resulting micelles was better than 85% when the ratio of polymer to CPT was 10:1. As a result, a 10:1 w/w ratio of polymer to drug was utilised to create micelles, which were then used to coat the membrane of RBCs in following experiments. Although CPTNV's encapsulation efficiency was just 1.9% when considering the nanoparticles' total mass, CPTNV-was Pmic's a staggering 11.6%. (Figure 4.3b). Thus, As drug partitioning in the former case also occurred inside the micellar core in addition to the lipid bilayer, CPTNV-Pmic was a superior carrier versus CPTNVE.

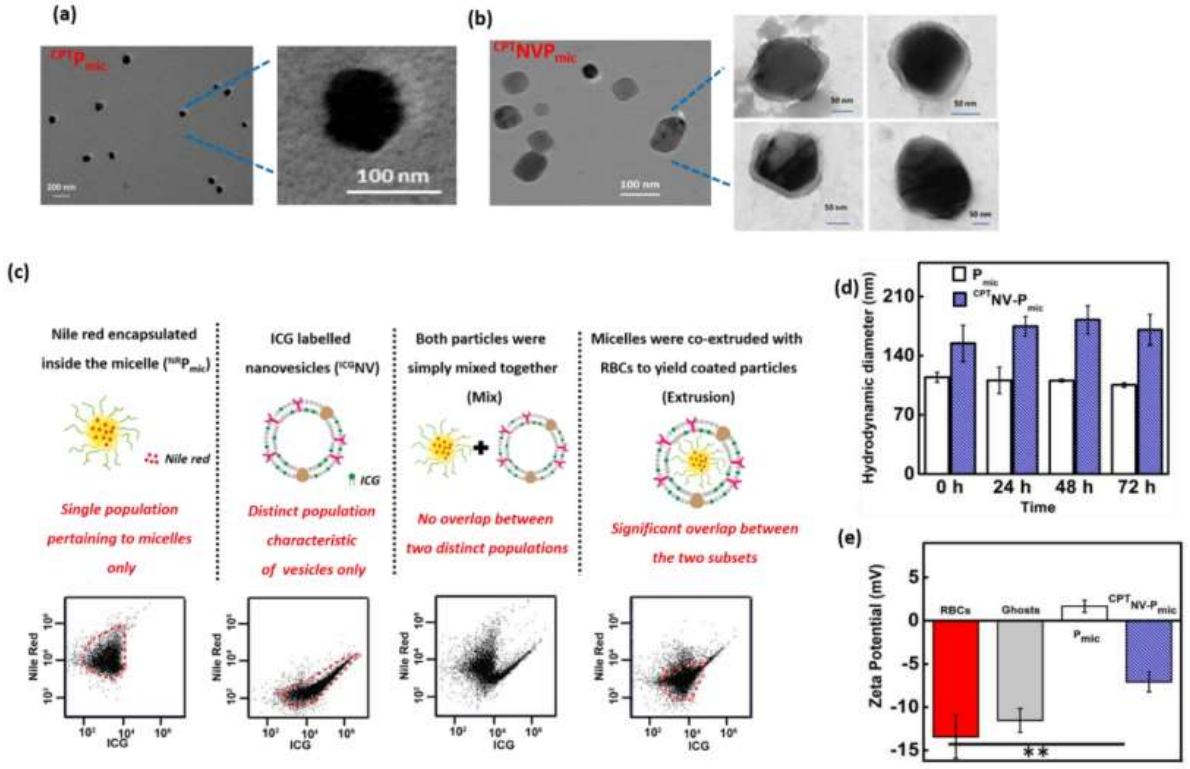
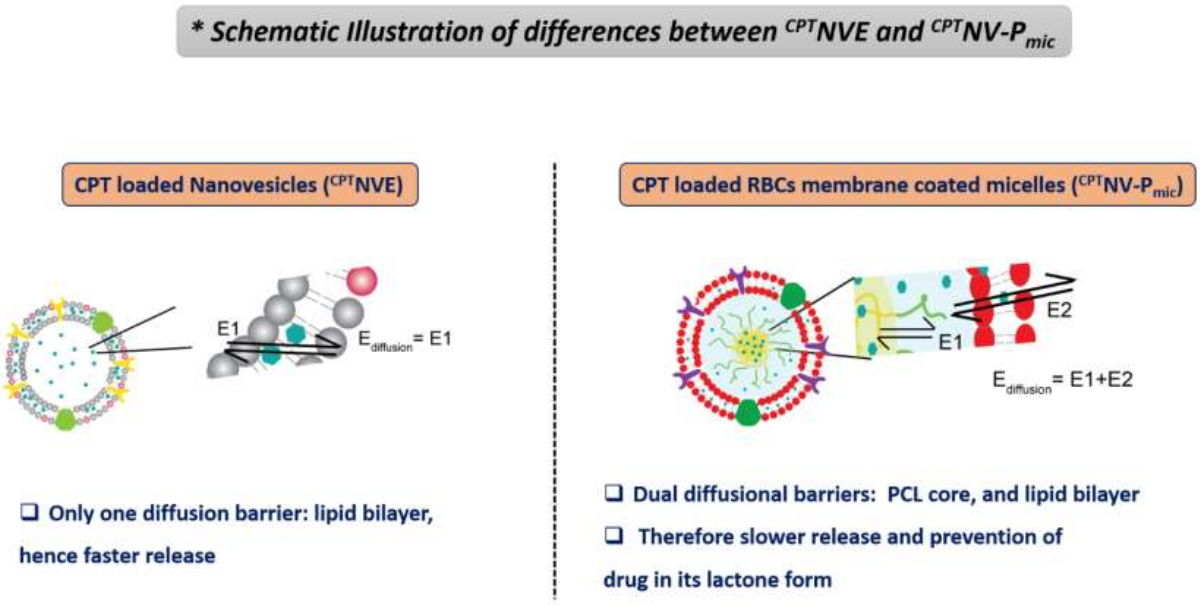


Figure 4.3: Hybrid nanovesicle physicochemical properties



Scheme 4.3: Example demonstrating the differences between CPTNVE and CPTNV-Pmic

In-vitro studies

Cell uptake and toxicity were investigated in murine lung cancer A549 cell lines after comprehensively defining red blood cell membrane-coated polymeric micelle nano drug carriers, and evaluating their release properties. Nile red, an insoluble in water dye, was loaded onto polymeric micelles and used in investigations of cellular absorption (NR). Fluorescent microscopy revealed small red patches in the cytoplasm of the cells, with the DAPI-stained nuclei appearing blue (Figure 3.4a). As the polymeric core encased the dye entirely, the bright cytoplasmic colour indicates efficient vesicle endocytosis.

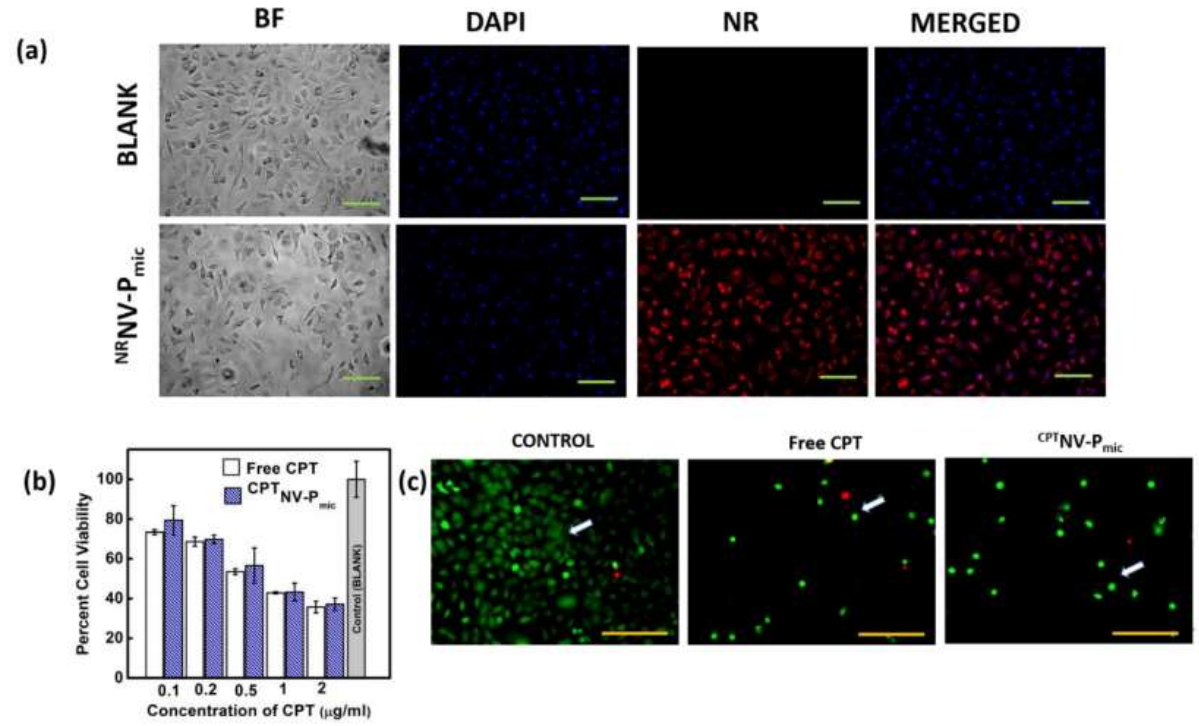


Figure 4.4: In-vitro cell studies.

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CONCLUSION

Free CPT was shown to be very toxic, causing extensive damage to the liver and kidneys, intestinal toxicity, and an inability to forestall cancer-induced weight loss due to cardiopulmonary dysfunction. Most importantly, it was easily flushed out of the body, which accounted for its weak tumor-suppressing and overall life-threatening effects. By first putting it into the polymeric micelle and then coating it onto the RBCs' membrane, its therapeutic potential may be considerably increased. PCL's hydrophobic core shielded CPT from hydrolysis, while RBC membrane coating added "stealth," both of which prolonged CPT's stay in the plasma and increased its tumour accumulation. Nanovesicles showed minor effects on the hepato-renal system and dramatically reduced lung metastases, albeit exhibiting some intestinal damage. The therapeutic usefulness of the reported nanovesicles may be further enhanced by modifying them to reduce the drug delivery system's intestinal toxicity. Hence, the nanovesicle platform described in this chapter is an improved version of the one mentioned in chapter 2, with a larger loading capacity to encapsulate CPT, considerably slower release kinetics, and substantially better control over drug hydrolysis. It has potential for future investigation as a component of dual treatment, with the goal of reducing the total drug dose while maintaining or improving therapeutic results compared to single chemotherapy.

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