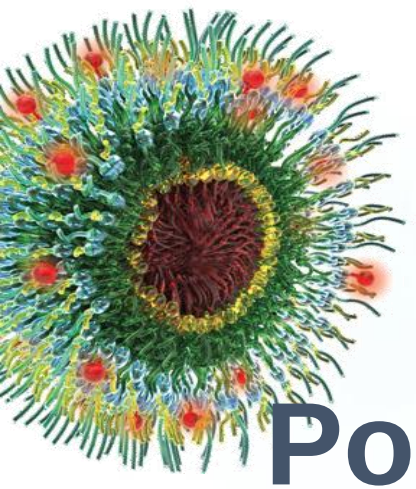
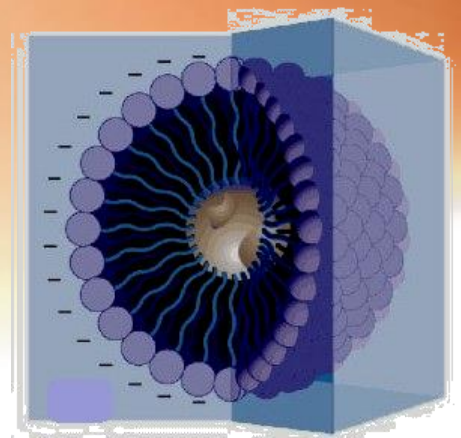


Polymeric micelle based formulation for delivery of docetaxel: Development and evaluation

Hema A. Nair, Megha Marwah



The Burden of Cancer Worldwide : A few figures and facts

- Cancer is among the leading causes of death worldwide.
- There were an estimated 14.1 million cancer cases around the world in 2014, of these 7.4 million cases were in men and 6.7 million in women.
- This number is expected to increase to 24 million by 2035.
- Among men, the 5 most common sites of cancer diagnosed include lung, prostate, colorectum, stomach, and liver.
- Among women the 5 most common sites diagnosed are breast, colorectum, lung, cervix, and stomach cancer.

Treatment options



Surgery



Radiation



Chemotherapy

Side effects of Chemotherapy

- Anaemia
- Nausea and vomiting
- Appetite Changes
- Bleeding
- Constipation
- Diarrhoea
- Fatigue
- Hair Loss
- Infection
- Infertility
- Mouth and throat changes
- Nervous system changes
- Pain
- Sexual changes
- Skin and nail changes
- Urinary, Kidney and Bladder changes

Docetaxel Trihydrate



- Docetaxel is a second generation antineoplastic agent belonging to the taxoid family.
- Acts by disrupting the microtubular network in cells that is essential for mitotic and interphase cellular functions.
- Efficacious in the treatment of cancers of breast and prostrate, non small cell lung carcinoma, gastric adenocarcinoma and head and neck cancers.
- The recommended therapy for docetaxel is 6 cycles given once every three weeks, each at the dose of 60 – 110 mg/m^2 administered iv over 1- 3 hrs.

Docetaxel trihydrate–a formidable challenge to formulation scientists due to poor water solubility

DRAWBACKS

Polysorbate 80 triggered hypersensitivity reactions necessitates use of steroids

Polysorbate 80 triggered accumulative fluid retention, resulting in weight gain, peripheral oedema and, occasionally, pleural or pericardial effusions

Incomplete treatment with docetaxel due to dose limiting toxicity

Lack of targeting leading to off-target toxicity

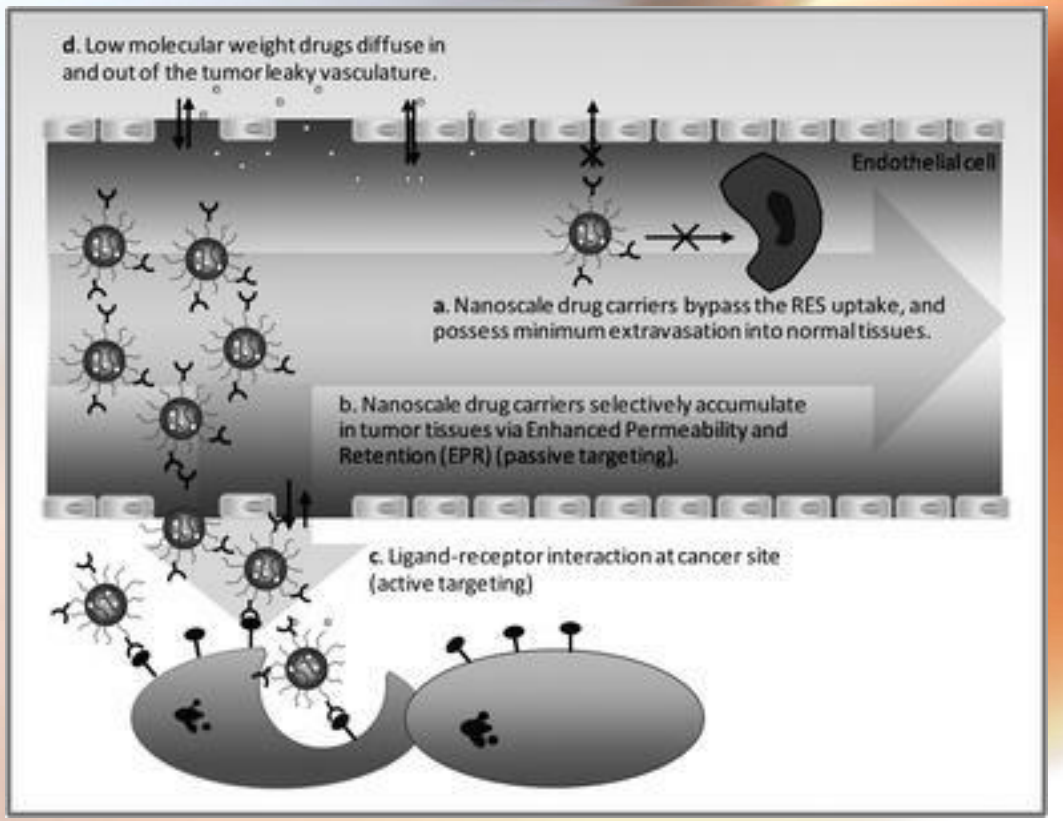
TAXOTERE®
Docetaxel solution made using Polysorbate 80

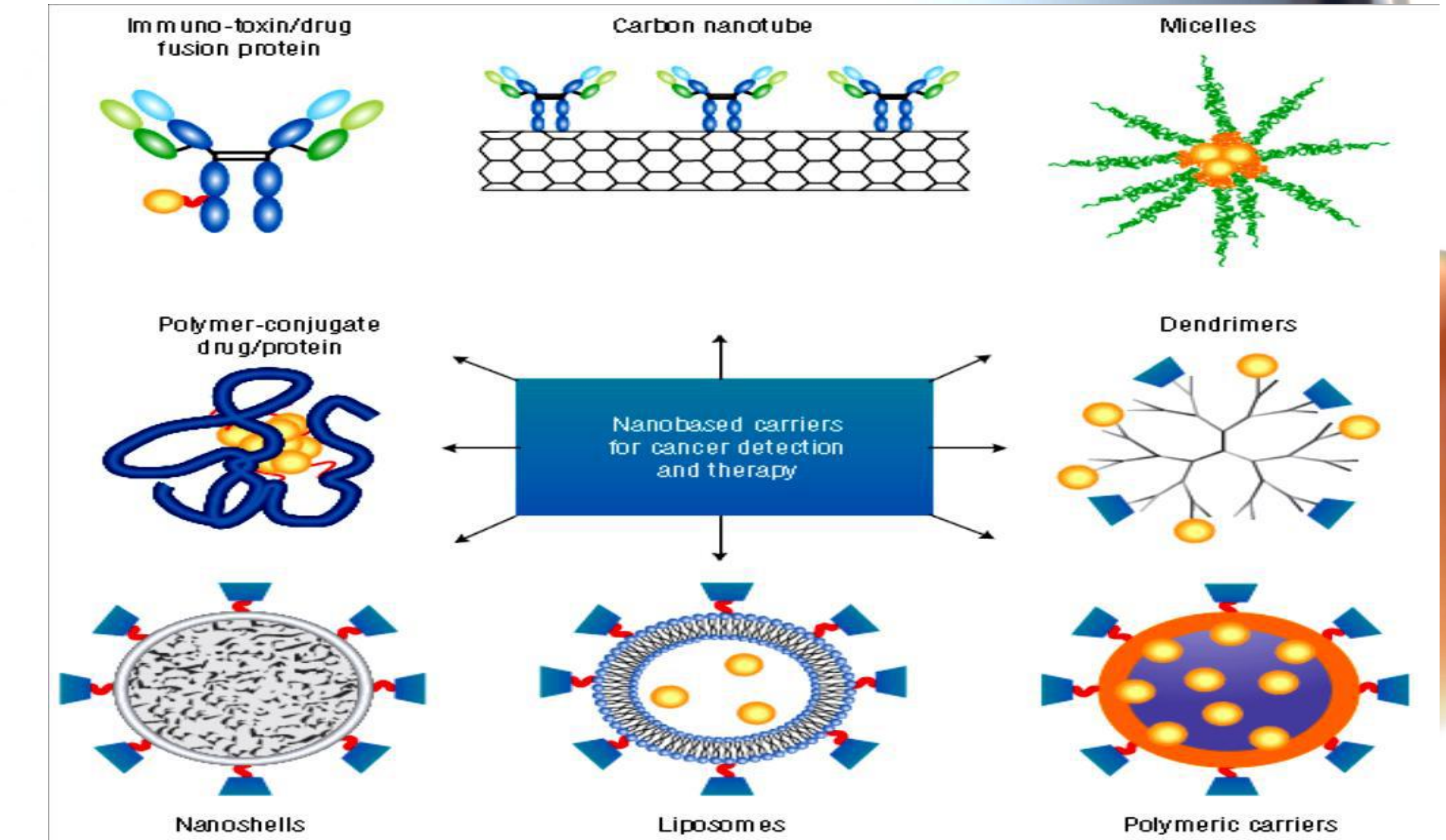


Several attempts at designing alternative vehicles including nanosystems for delivery of docetaxel

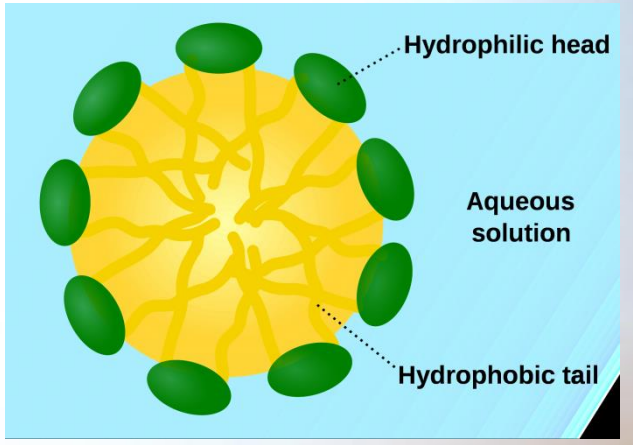
Why nanosystems

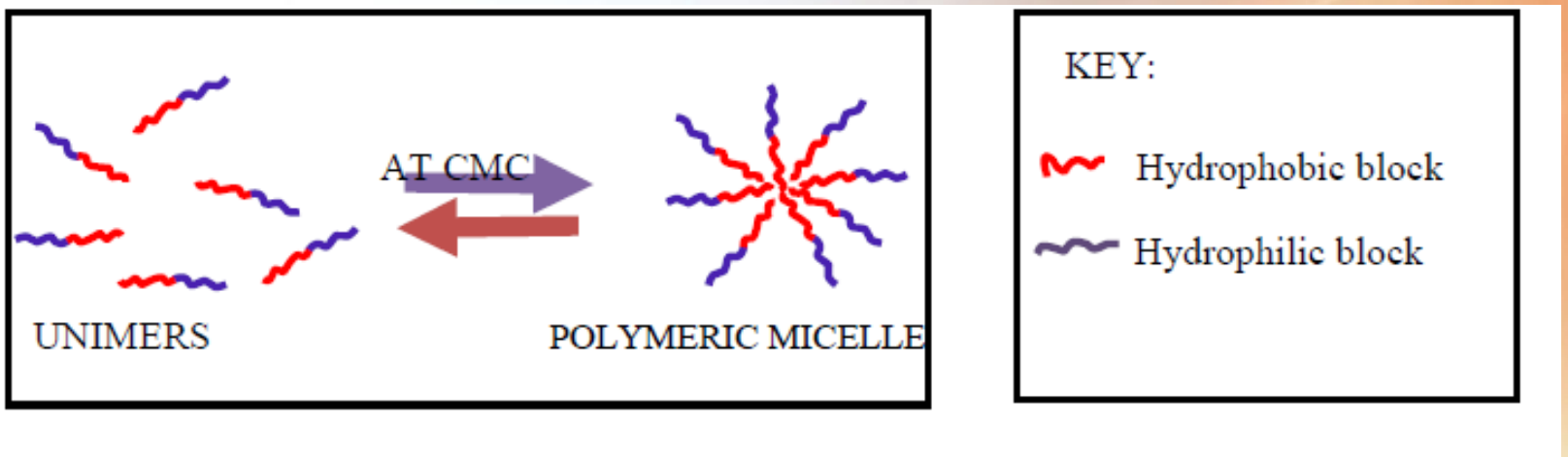
- ✓ Achieve targeting, thereby reducing the concentration at nonspecific sites and decreasing systemic toxicities associated with the same.
- ✓ Improve solubility of hydrophobic drugs thereby facilitating their parenteral administration.
- ✓ Has potential to provide with controlled release aiding in maintaining constant therapeutic dose at site of action.
- ✓ Can also help increase half-life of drug by reducing its clearance.
- ✓ Can serve to increase drug stability by protecting it from the harsh invivo environment.
- ✓ Nanotechnology also helps accomplish drug delivery across the blood brain barrier (BBB).



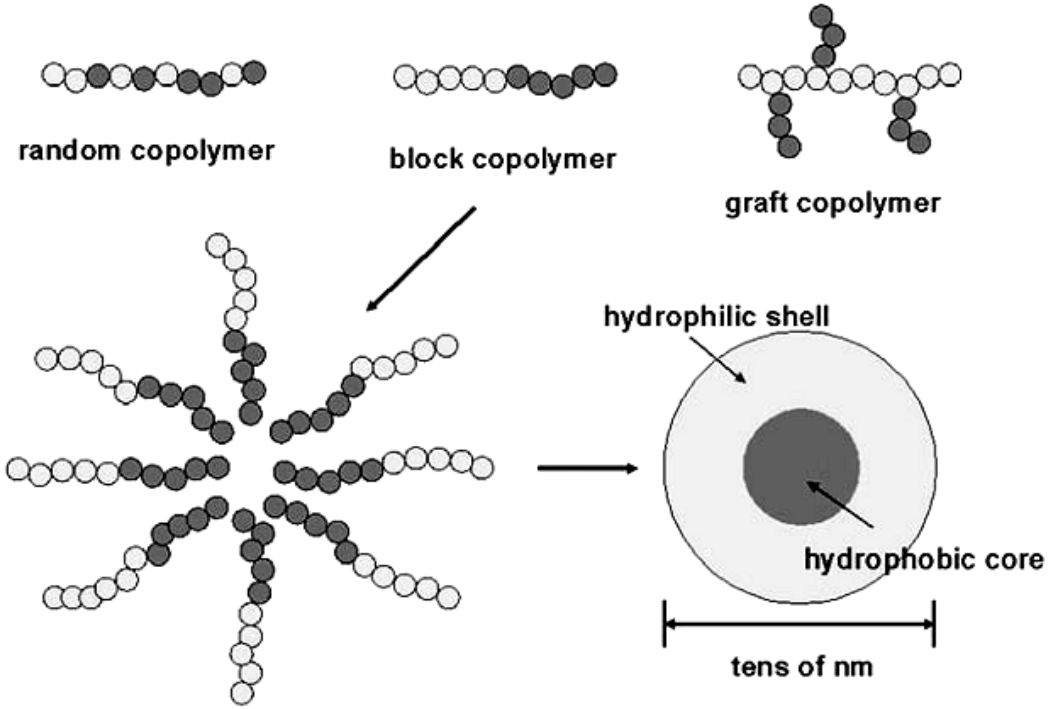

Immuno-toxin/drug fusion protein
Carbon nanotube
Micelles
Polymer-conjugate drug/protein
Nanobased carriers for cancer detection and therapy
Dendrimers
Nanoshells
Liposomes
Polymeric carriers

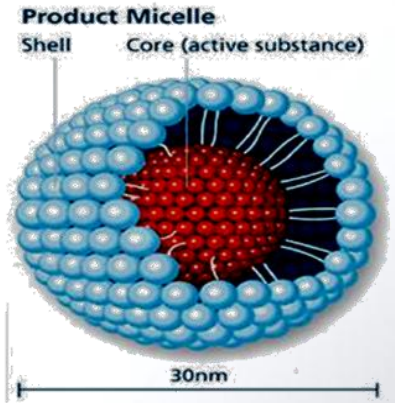
ALTERNATIVE : POLYMERIC MICELLAR SYSTEMS

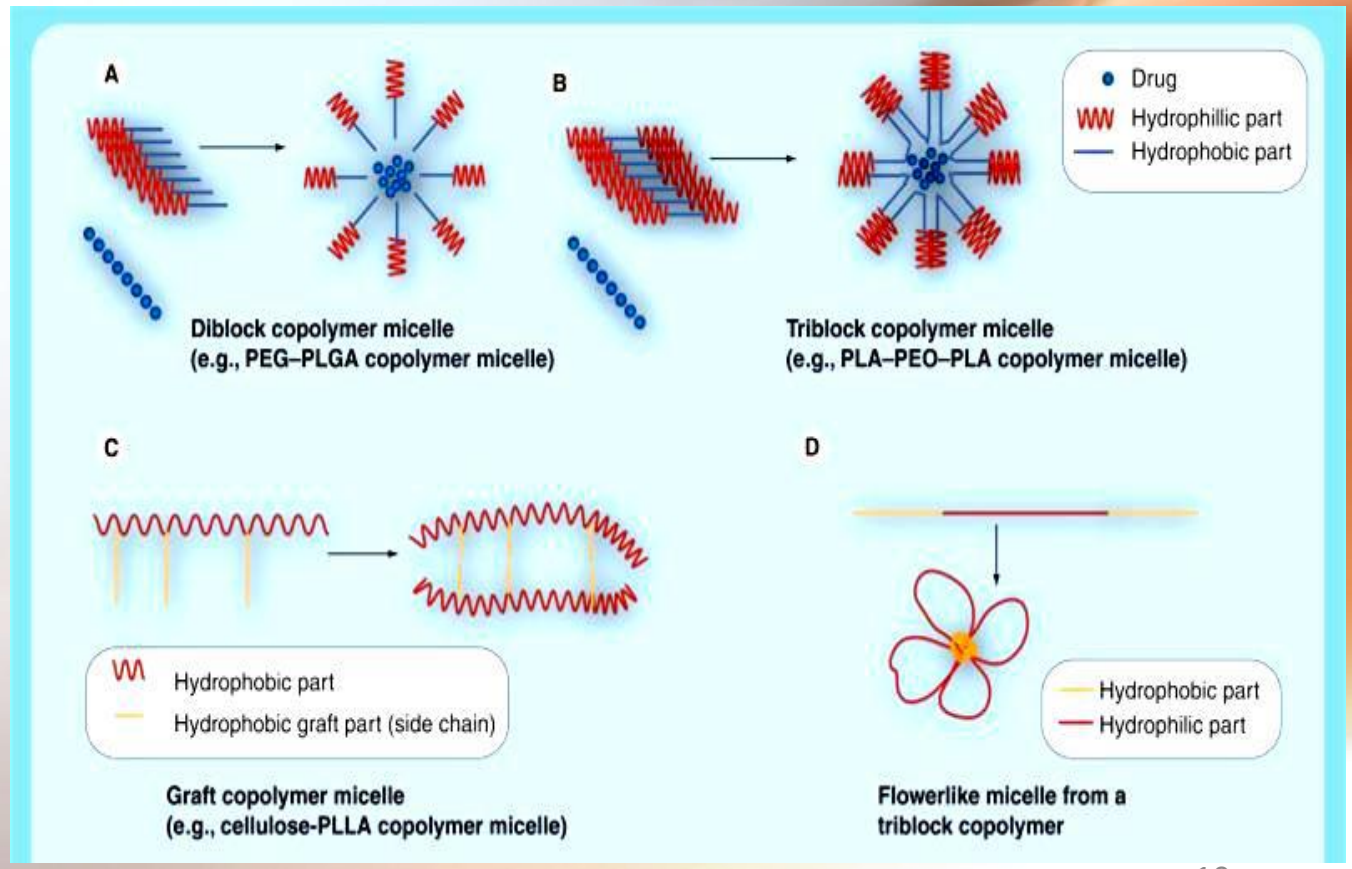




Polymeric Micelles







Why Polymeric micelles:

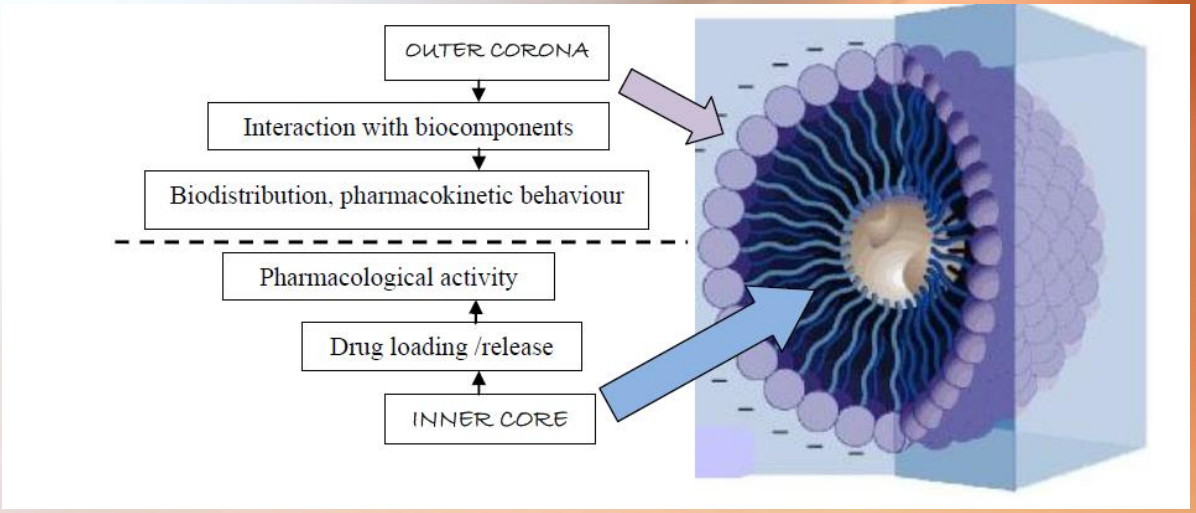
***Small size*: The size of polymeric micelles is usually in the range of 10 to 200nm.**

Solubilisation of hydrophobic drugs

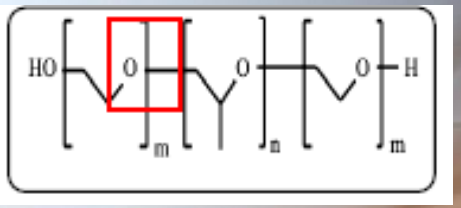
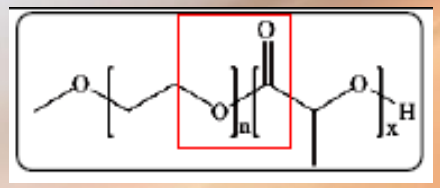
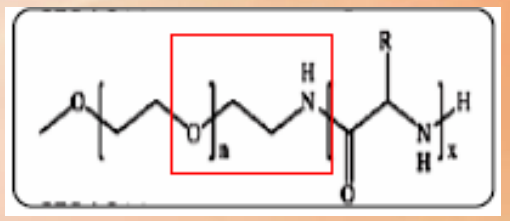
Cargo protection

Separated functionality

Low toxicity

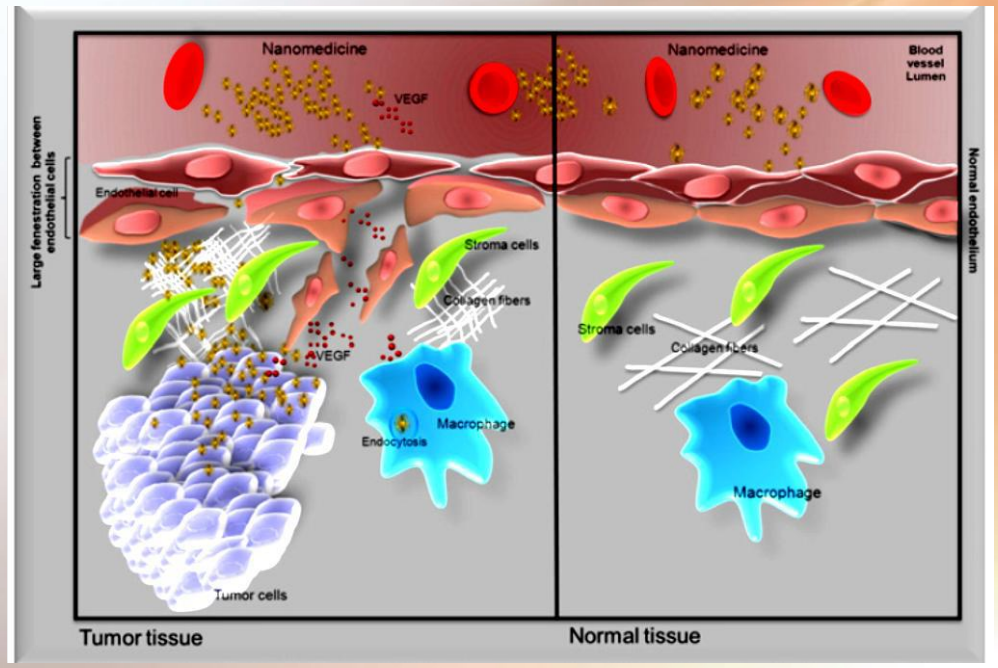


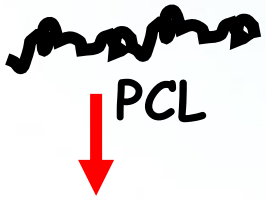
Low CMC : withstand dilution in body fluids

CHEMICAL CLASS, CATEGORY OF COPOLYMERS	EXAMPLES	GENERAL STRUCTURE
1. Polyether PEO-PPO	Pluronic F68, F127, P105.	
2. Polyester a.PEG-Polylactides b.PEG-Polylactones.	a. PEG-PDLLA, PEG-PLGA b. PEG-PCL	
3. Polyaminoethers PEG-Polyaminoacids Examples:	PEG-PAsp, PEG-PHis	

OBJECTIVE OF STUDY: To synthesize biodegradable amphiphilic POLYETHYLENE GLYCOL – POLYCAPROLACTONE copolymers and employ the same for development of micelle based nanocarrier system for docetaxel offering following advantages over existing therapy:

- ✓ **Use of biodegradable polymers in aqueous vehicle in place of polysorbate 80**
- ✓ **Patient compliant treatment**
- ✓ **Small size of micelles confers passive targeting abilities through Enhanced Permeation and Retention Effect**





PCL





PEG



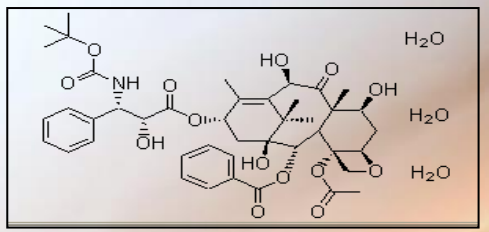
PEG-PCL copolymer

Crystallinity and hydrophobicity- barriers to biodegradability

Reduced crystallinity and hence improved biodegradability

Above CMC





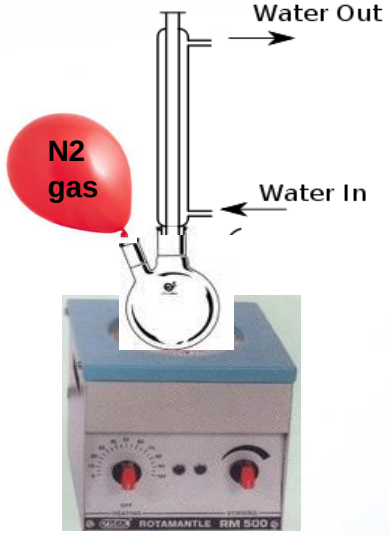
DOCETAXEL TRIHYDRATE



Drug loaded Polymeric Micelle

IMPROVED SOLUBILIZATION
TARGETING POTENTIAL

SYNTHESIS OF Polyethylene Glycol- Polycaprolactone (PEG-PCL) BLOCK COPOLYMERS



mPEG2000 +

Caprolactone monomer

+ catalyst at 160°C

Stannous Octate

I

II

III

IV

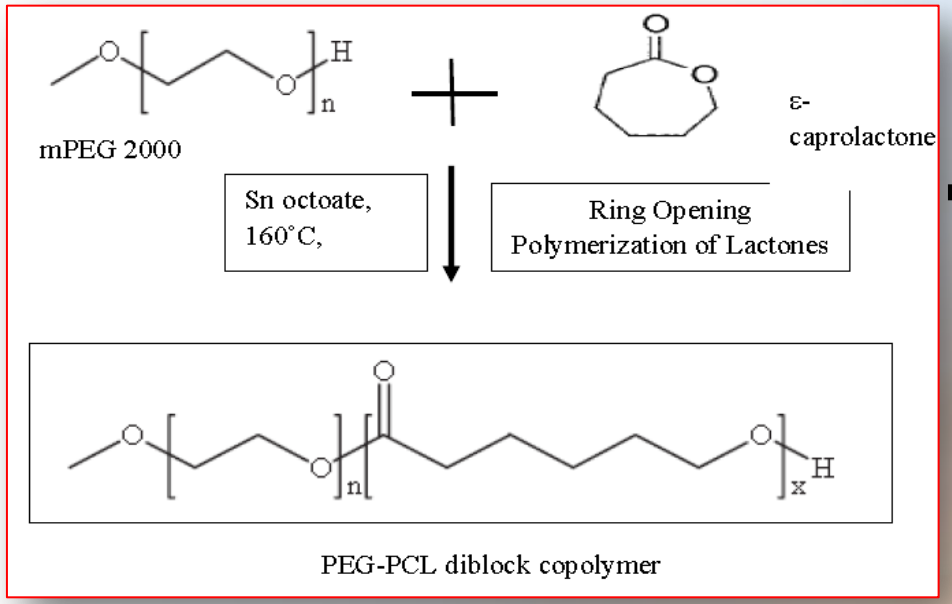
PEG-PCL linear diblock copolymers

➢Block copolymers employing four different ratios of PEG: Monomer (ε-caprolactone) were synthesized by **ring opening bulk polymerization** reaction.

➢Completion of reaction was monitored by Thin Layer Chromatography (TLC).

CHARACTERISATION OF SYNTHESIZED BLOCK COPOLYMERS:

1. IR spectroscopy for identification



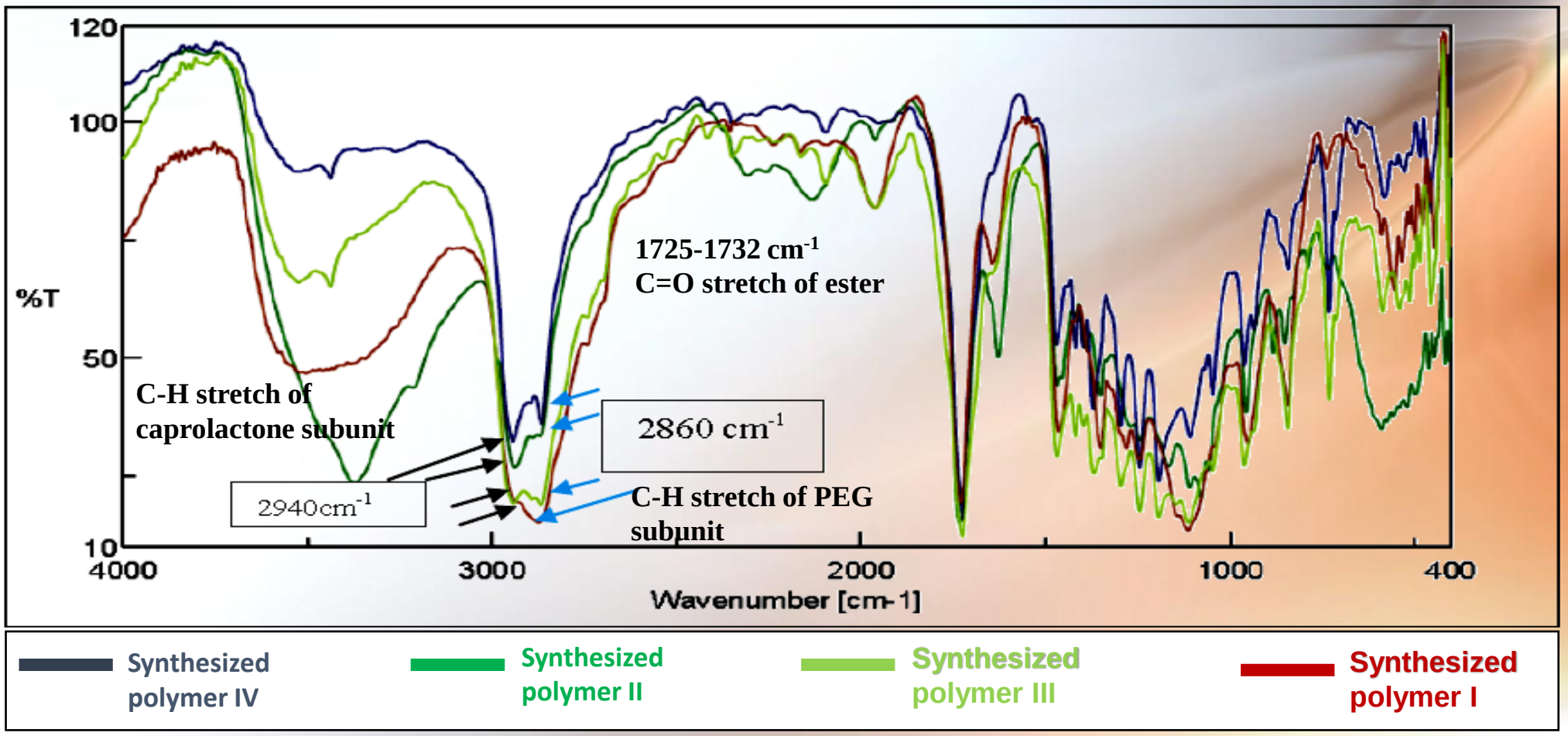
4. XRD for determination of crystallinity

3. Critical Micelle Concentration (CMC): Pyrene Fluorescent probe method

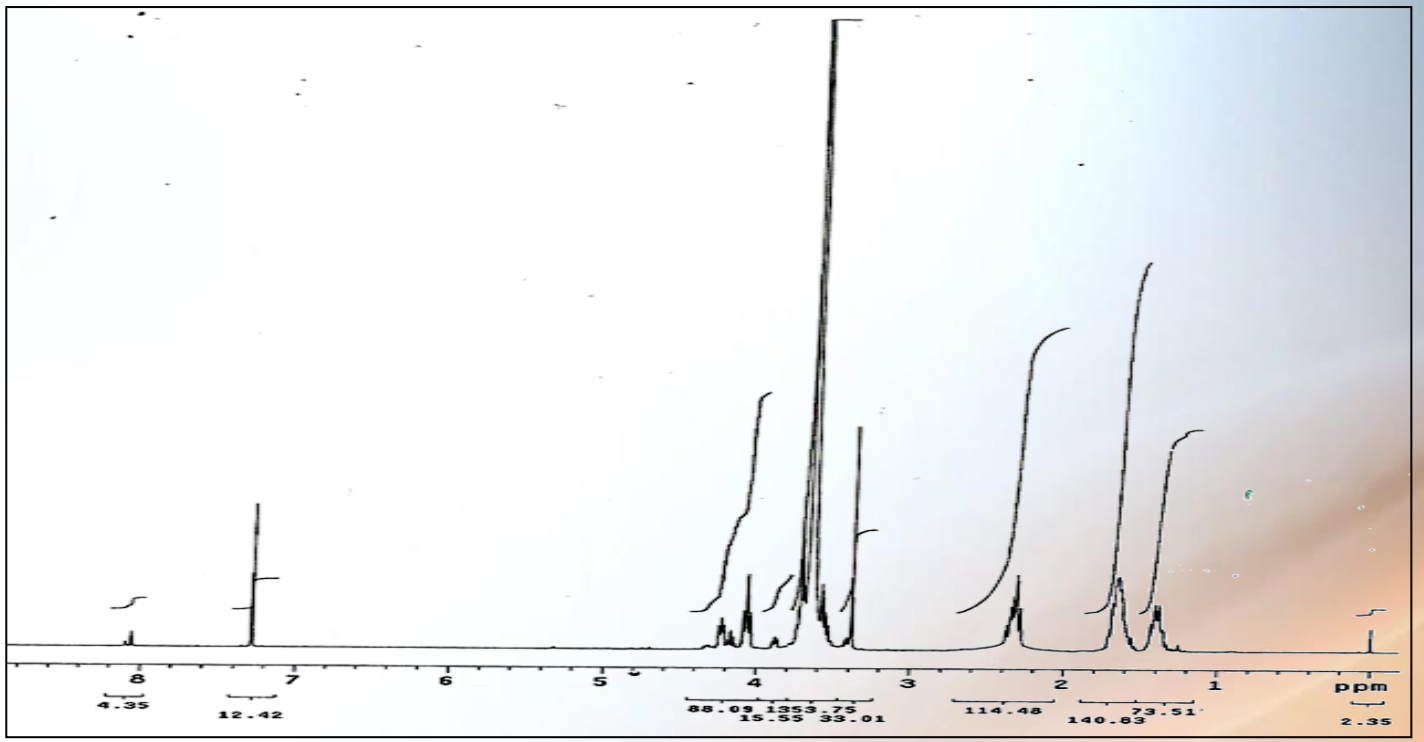
2. H1-NMR spectroscopy for number average molecular weight determination

CHARACTERISATION OF SYNTHESIZED BLOCK COPOLYMERS:

1.Identification by Infrared Spectroscopy :



2. Identification and Determination of Number Average Molecular Weight (Mn) and HLB by H1-NMR Spectroscopy: Mercury Plus 300 MHz NMR Spectrometer, VARIAN, USA

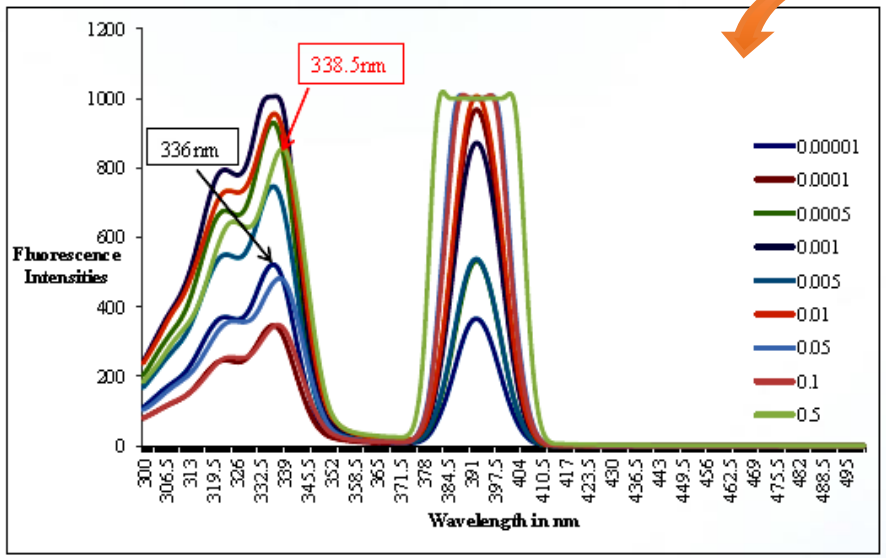


HLB= 20X (MnPEG/ MnCopolymer)

POLYMER	I	II	III	IV
Mn PEG	1430.50	1329.51	1556.70	**1557.36**
Mn $_{PCL}$	432.69	882.46	2049.49	**2979.73**
Mn $_{copolymer}$	1863.2	2211.98	3606.19	**4537.09**
HLB	15.35	12.02	8.632	**6.86**

CHARACTERISATION OF SYNTHESIZED COPOLYMERS:

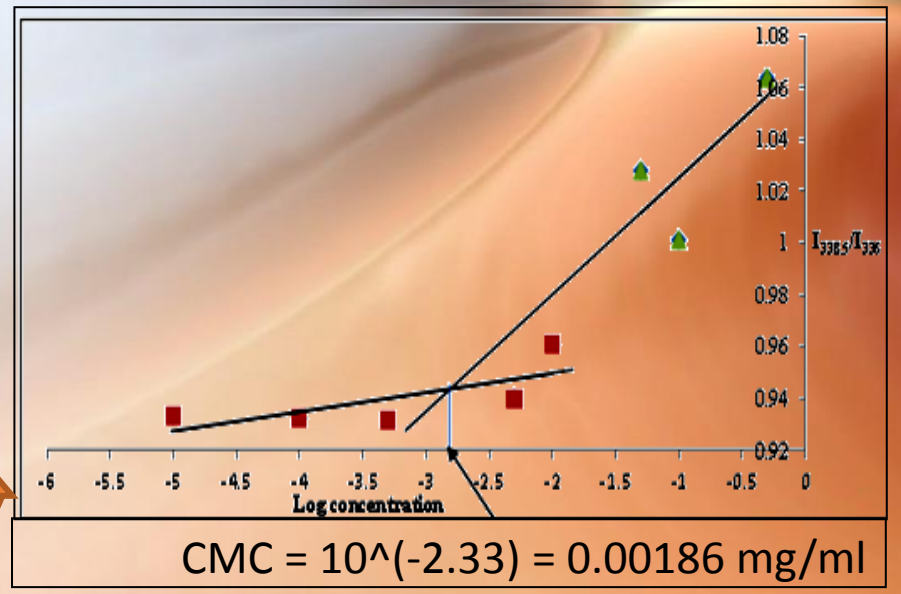
3. Determination of CMC : Pyrene fluorescent probe method



Excitation spectra of pyrene in Polymer IV at different concentrations

And

Plot of I_{339}/I_{336} versus log concentration

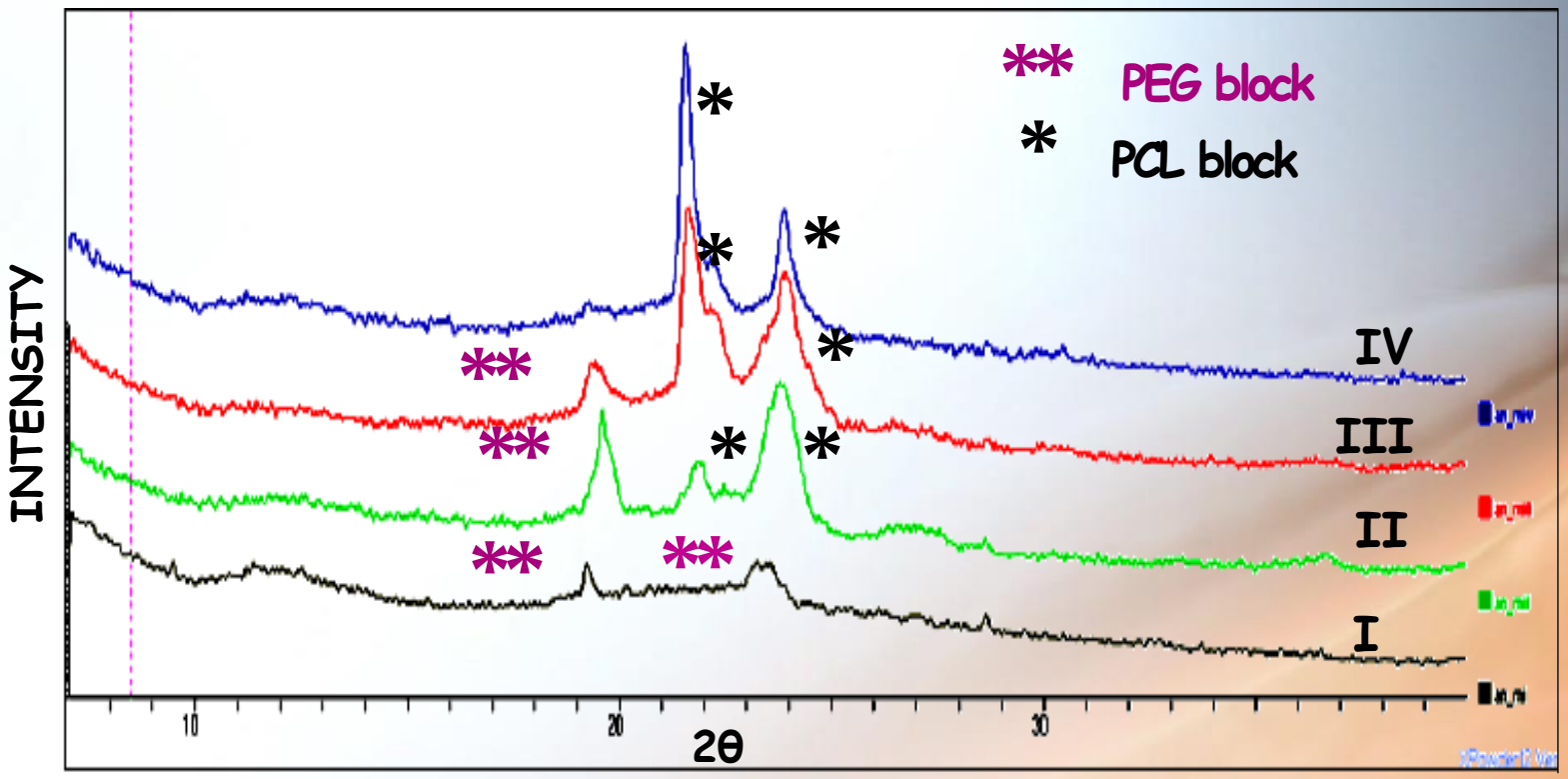


CMC = 10^(-2.33) = 0.00186 mg/ml

POLYMER	I	II	III	IV
CMC (mg/L)	22.38	12.5	2.81	**1.86**

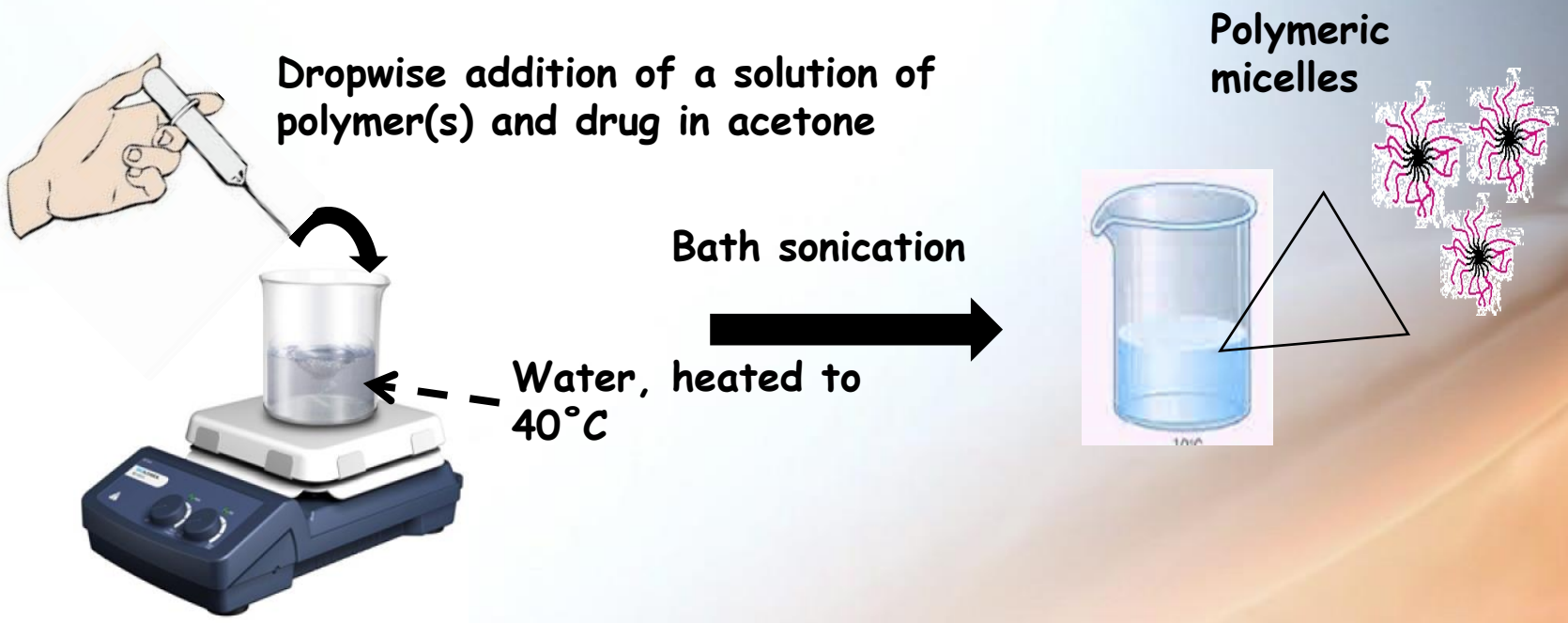
4. X-Ray Diffraction analysis of synthesized copolymers:

Philips P Analytical X'pert Pro X ray diffractometer



- Peaks at 19˚ and 23˚: correspond to PEG block
- Peaks at 22˚ and 24˚: correspond to PCL block
- PCL block crystallinity increases and PEG block crystallinity decreases across polymers I through IV.

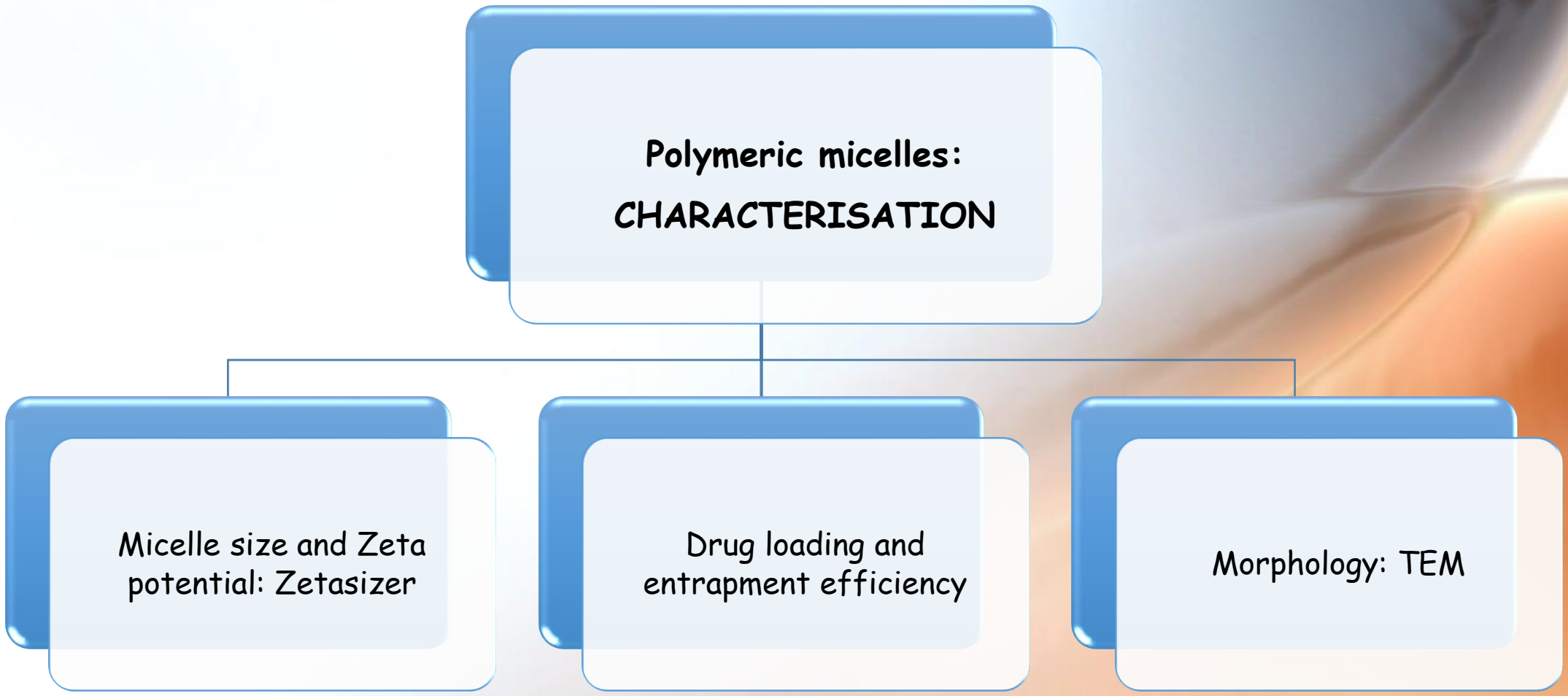
FORMULATION OF MICELLAR SYSTEMS



Micellar dispersions were subjected to lyophilization in presence of **hydroxypropyl beta cyclodextrin (HPBCD)** as cryoprotectant.

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CHARACTERISATION OF MICELLAR DISPERSIONS

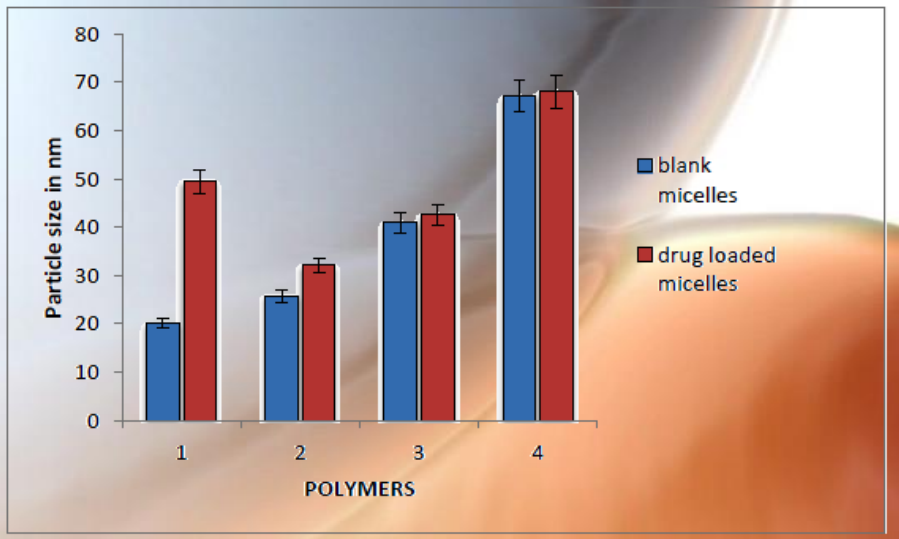


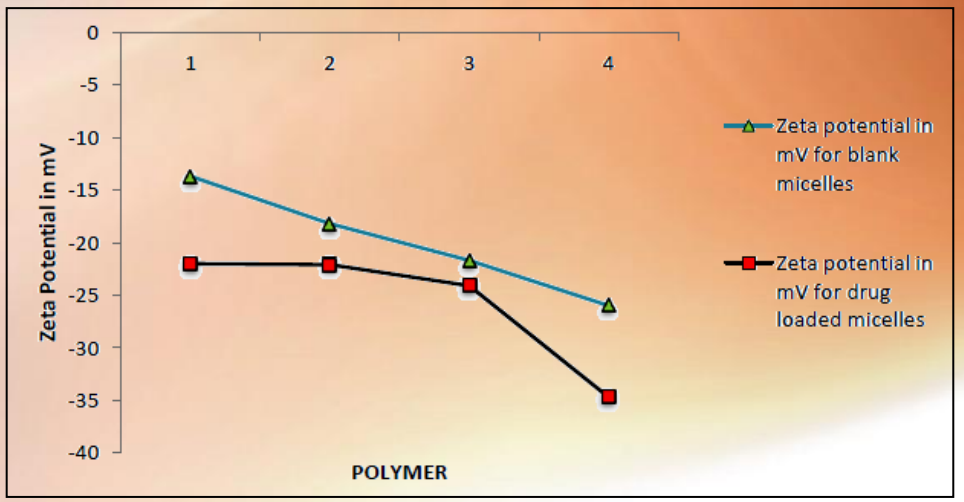
EVALUATION OF MICELLAR DISPERSIONS

1. Micelle Size and Zeta Potential:

Zetasizer Nano ZS (Malvern)

POLYMER/ PARAMETER		I	II	III	IV
Micelle Size (in nm)	BLANK	19.98	25.8	40.95	**67.11**
	DRUG LOADED	49.49	32.05	42.52	**68.03**
Polydispersity Index (PI)	BLANK	0.409	0.404	0.413	**0.308**
	DRUG LOADED	0.499	0.479	0.389	**0.209**
Zeta Potential (in mV)	BLANK	-13.7	-18.2	-21.7	**-26**
	DRUG LOADED	-22.0	-22.1	-24.1	**-34.7**





2. Drug loading and entrapment efficiency of simple and mixed micelles:

Micellar dispersion – Dilution and Analysis

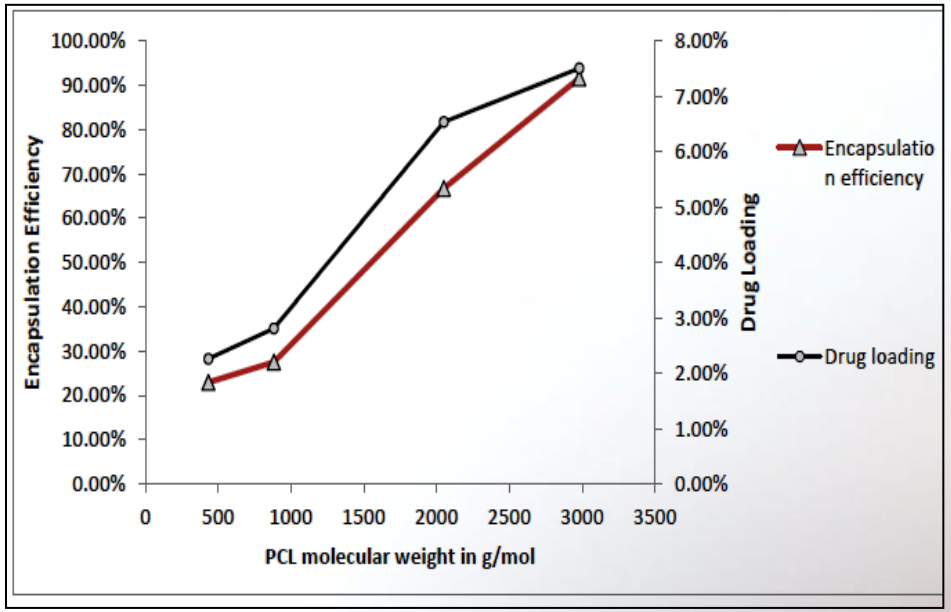
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Centrifugation

↓

Dilution and Analysis of supernatant

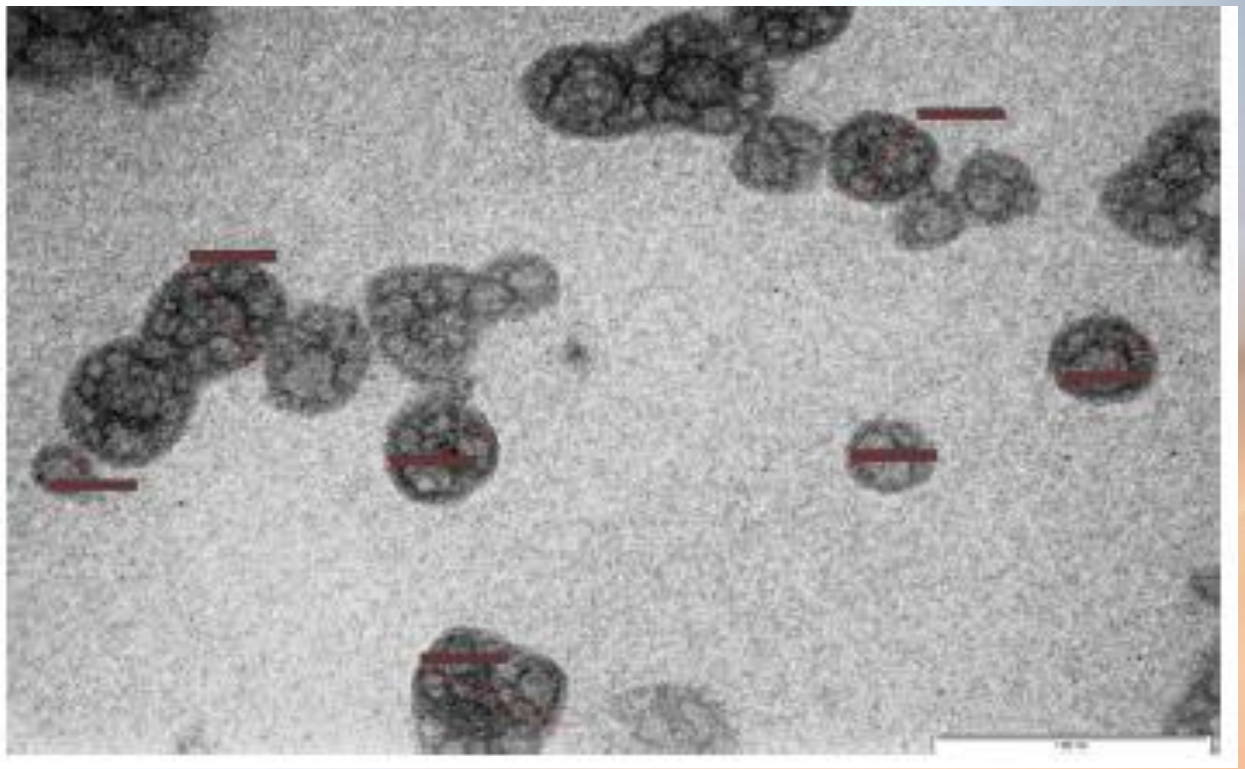
POLYMER/ PARAMETER	I	II	III	IV
Drug loading (% w/w)	2.25	2.8	6.54	7.51
Entrapment Efficiency (%w/w)	22.92	27.48	66.74	91.64
HLB	15.35	12.02	8.632	6.86



Drug loading and encapsulation efficiency of micelles formulated from synthesized polymers

3. Morphology Of Micelles: TEM

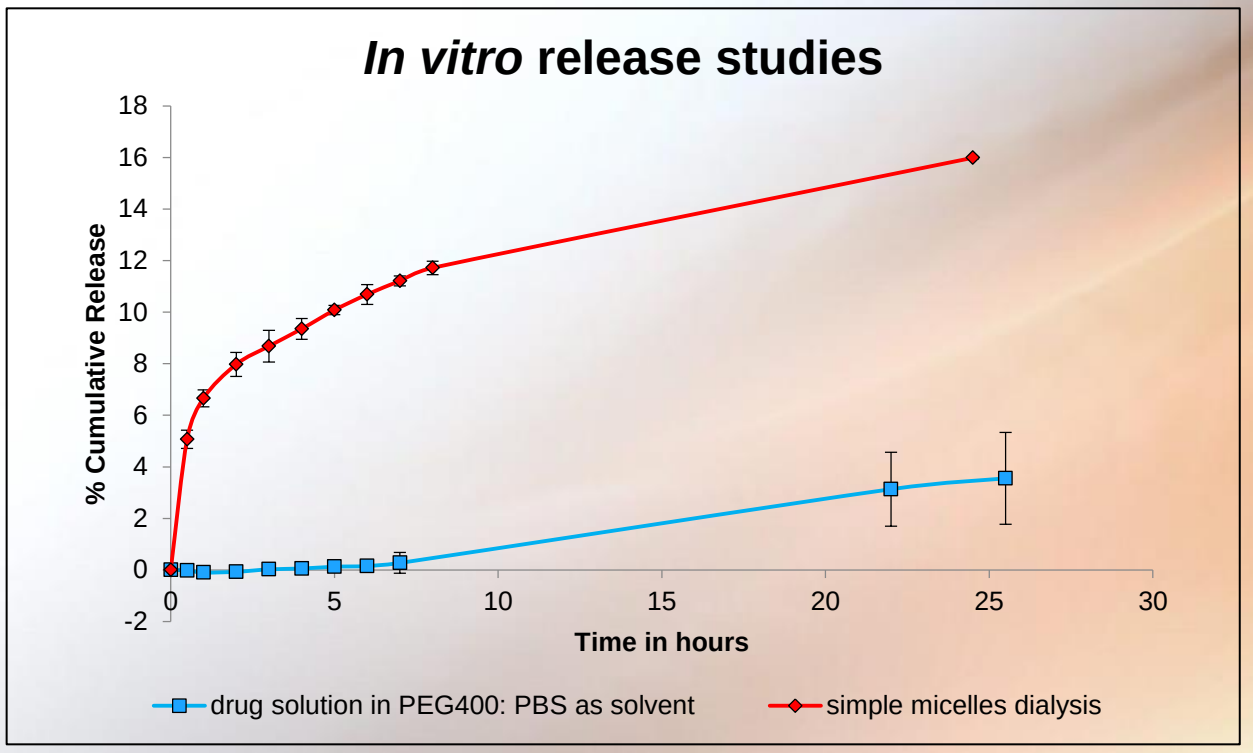
Transmission Electron Microscope (Philips CM200)



TEM IMAGE OF MICELLES PREPARED USING POLYMER IV (Scale: 100nm)

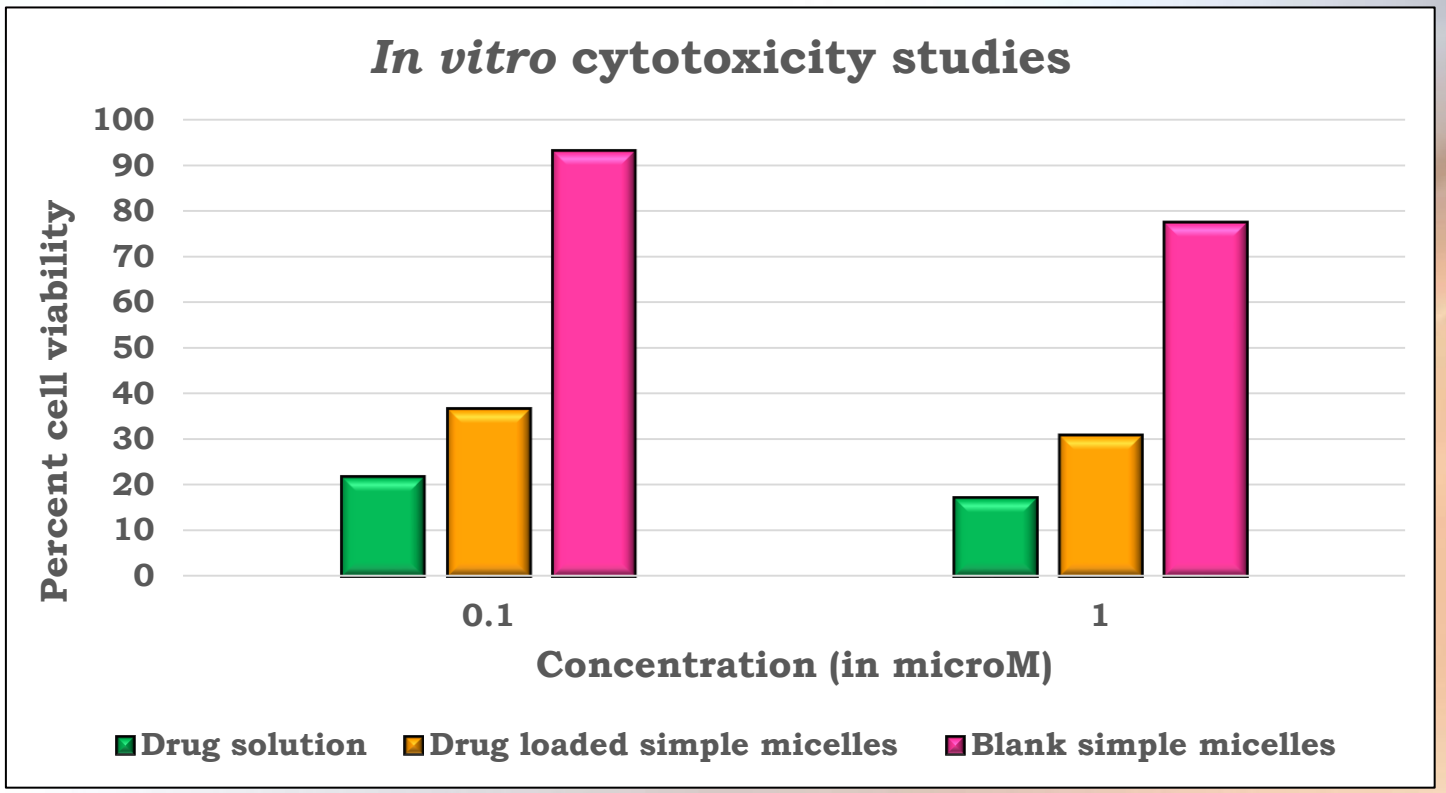
IN VITRO RELEASE STUDIES: SIMPLE AND MIXED MICELLES

- ✓Micellar Dispersion/ Drug solution in dialysis bag MWCO: 12000Da
- ✓Receptor medium: PEG 400 : PBS ; 75 : 25 v/v
- ✓Gentle stirring at 37°C on a stirring mantle
- ✓Aliquotes analysed by validated HPLC method



IN VITRO CYTOTOXICITY STUDIES

- A 549 cell line : Adenocarcinomic human alveolar basal epithelial cells
- Sulforhodamine B (SRB) assay for viable cells



SUMMARY

- Amphiphilic block copolymers ⟶ tailored to suit needs.
  - Extremely low CMC values capable of withstanding dilution in bloodstream possible.
- Small size imparts passive targeting properties (Enhanced Permeation and Retention Effect).
  - Polymeric micelles containing biodegradable ester linkage have the potential to avert side effects vis-à-vis Taxotere® (marketed drug solution of docetaxel).
- Polymeric micelles hold good potential for delivery of cytotoxic agents.

ACKNOWLEDGEMENTS

Acknowledgements:

• We would like to thank Dr. Juvekar and Mr. Subroto at ACTREC for helping us with cell line work.

•We would also like to express our gratitude towards Mrs. Nutan Agadi at IIT for conducting NMR studies, Glenmark for DSC analysis, Mr. Nilesh Kulkarni at TIFR for XRD studies.

•Special thanks to Mr.Milind Tikle at Dept. of Chem., Mumbai University for helping with spectrofluorimetric analysis for determination of CMC.

Potential for vaccine delivery?

KEY FEATURES OF POLYMERIC MICELLAR SYSTEMS WHICH MAKE THEM SUITABLE FOR VACCINE DELIVERY:

- Due to their small size (generally <100 nm) they particularly facilitate the antigen delivery to antigen presenting cells (APCs), such as dendritic cells (DCs) in the draining lymph nodes.

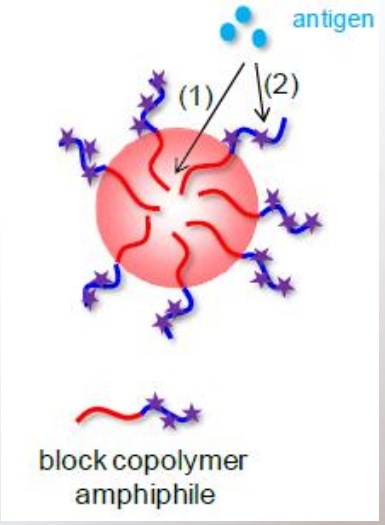
- Micelles are capable of targeting DCs by traveling through lymphatics directly to lymph nodes, promoting germinal centre formation

- They can also easily display suitable surface properties (nature, surface charge) through the appropriate choice of biocompatible hydrophilic segments of the micelle corona.

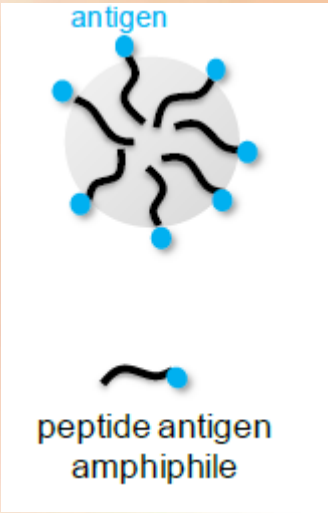
- Through appropriate chemical design of the hydrophobic and hydrophilic blocks (presence of reactive groups, cationic moieties,…), a variety of additional immunostimulatory molecules (such as Toll Like Receptor (TLR) ligands, mannose receptor ligands,…) can be easily incorporated in these systems in a controlled fashion, to induce an enhanced activation of the DCs.

Two main types of micellar nanocarriers for vaccine delivery

Amphiphilic block copolymers used for self-assembly into micelles while an antigenic peptide is associated to the latter through either encapsulation or surface coupling



Peptide amphiphile self-adjuvant system, wherein the antigenic peptide is used as the hydrophilic head-group and covalently bound to a hydrophobic moiety (typically alkyl tail) for self-assembly into micelles



A few Studies……POLYMERIC MICELLAR SYSTEMS

Polymeric micellar system	Antigen	Finding
PEG-b-poly(propylene sulfide) block copolymer	Ovalbumin	2.4-fold OVA-specific CD8+ T cells in the blood and higher (1.7-fold) interferon gamma levels from splenocytes upon restimulation than in mice immunized with free OVA with CpG as adjuvant.
Cationic polyethyleneimine (PEI) – stearic acid based micelles	Hydrophobic melanoma peptide antigen Trp2	Trp2-loaded micelles accumulated preferentially in the medulla and paracortex of the draining lymph nodes and were present at negligible levels in the systemic circulation. Mice immunized with Trp2-loaded micelles showed significantly higher Trp2-specific cytotoxic T lymphocyte activity than mice immunized with free Trp2 or a mixture of Trp2 and empty micelles.
Micelles based on chitosan modified with hydrophobic phenyl alanine bearing mannose moieties – for triggering mannose-receptor mediated endocytosis in APCs.	Plasmid DNA encoding hepatitis B surface antigen (pHBsAg)	Enhanced cell uptake (RAW 264.7 and DC 2.4 cells) and high *in vitro* transfection efficiency Intradermal immunization of BALB/c mice indicated that polyplexes with the plasmid DNA encoding pHBsAg induced significantly higher serum antibody titer in comparison to naked pHBsAg

PEPTIDE MICELLAR SYSTEM

Pepetide based micellar system	Finding
Peptide epitopes selected from the HSV envelope glycoproteins B and D (gB and gD), that had their N-terminus modified with hydrophobic moieties containing a double C18 alkyl chain	The cytokine production triggered by the gB and gD micelles in mouse (RAW 264.7) and human (U937) macrophages increased compared to the production triggered by the pure gB and gD peptides

THANK YOU

FOR

PATIENT

LISTENING