CHAPTER 4

MATERIALS & INSTRUMENTS

This chapter includes a list of all materials and the instruments used for characterizations purpose. A brief information about the drug; doxorubicin hydrochloride and materials; gellan gum, poly(ethylene glycol) and sophorolipid are mentioned in this chapter.

MATERIALS AND INSTRUMENTS

4.1 Materials

4.1.1 Drug

- **Doxorubicin hydrochloride**

  Gift sample from Sun Pharmaceuticals Industries Ltd Baroda, India.

4.1.2 Polymers and biosurfactant

- **Gellan gum**

  Gift sample from CP Kelco,USA.

- **Polyethylene glycol (PEG-SH)**

  Rapp Polymere, Tubingen, Germany.

- **Sophorolipid**

  Synthesized at Hoffkins Institute, Lower parel, Mumbai, India.

4.1.3 Chemicals and Reagents

- Chloroauric acid : Sigma-Aldrich, USA
- Texas red : Sigma-Aldrich, USA
- DMEM (cell culture media) : Gibco, USA
- Phosphate buffered saline : Himedia, India
- Paraformaldehyde : Sigma-Aldrich, USA
- 4'-6-diamidino-2-phenylindole : Molecular probes, USA
- 1,4-diazobicyclo-2,2,2-octanex : Sigma-Aldrich, USA
- MTT - tetrazolium salt : Sigma-Aldrich, USA
- Sodium hydroxide : Merck, India
- Hydrochloric acid : Merck, India
- Sodium chloride : Merck, India
- Dimethyl sulfoxide : Sigma-Aldrich, USA
- Luria Broth : Himedia, India
- Agar powder : Sisco Research Laboratory, India

4.2 Instruments

- UVA/is/NIR dual beam spectrophotometer, Jasco V 570, Japan.
- Transmission electron microscopy, TECHNAI G2 F30 S-TWIN Instrument, Netherlands.
- X- Ray diffraction, PANalytical Xpert PRO Instrument, USA.
- Fourier-Transform Infrared spectrophotometer, Perkin Elmer Instruments, USA.
- Atomic absorption spectrophotometer, AA 201, Chemito, India.
- Fluorescence spectrophotometer, Cary Eclipse, Varian, USA.
- Zeta potential analyzer, Brookhaven Instruments Corporation, USA.
- Nihon Kohden fully automatic haematology analyzer, MEK 6410, Japan.
- Semi automated clinical chemistry analyzer, Microlab 300, Vital Scientific, Netherlands.
- Electrolyte analyzer, model 9180, Roche, USA.
- Confocal microscope, Zeiss LSM 510, Germany.
- Light microscope, Nikon Eclipse 50i, Japan.
- Cryostatic bath, Haake Phoenix C25P, Germany.
- Spray dryer, Jay Instruments and Systems Private Limited, India.
- Viscotech Rheometer, Reologica Instruments AB, Lund, Sweden.
- Incubator, Thermo Electron Corporation, USA.
- Bio-Rad microplate reader, Heraeus, USA.
- Culture hood, Bio-Air, Italy.
- Centrifuge, Biofuge Stratos, Heraeus, Germany.
- Centrifuge, Eppendorf, USA.
- pH meter, Eutech instruments, Singapore.
- Orbital shaking incubator, REMI, India.
- Digital balance, Shimadzu, Japan.

4.3 Softwares

- Origin 6.1, USA.
- Gatan software, USA.
- GraphPad Instat, USA.
- Confocal image analyzer, Germany.

4.4 Drug profile

4.4.1 Doxorubicin hydrochloride

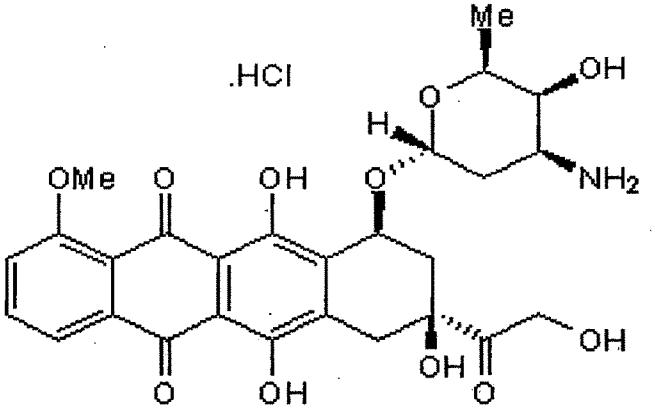
Synonyms: Adriamycin®, Doxil®, 14-Hydroxydaunomycin HCl.

Description: Doxorubicin hydrochloride is a cytotoxic anthracycline antibiotic isolated from a culture of streptomyces peucetius var. caessius or by chemical synthesis from daunorubicin.

Appearance: Orange-red crystalline powder.

CAS number: [25316-40-9],

Structural formula: The chemical name of doxorubicin HCl is (8S,10S)-10-(4- amino-hydroxy-6-methyl-tetrahya'ro-2H-pyran-2-yloxy)-6,8,11-trihydroxy-8-(2- ydroxyacetyl)-1-ethoxy-,8,9,10-tetrahydrotetracene-5,12-naphthacenedione hydrochloride.



Molecular formula: $C_{27}H_{29}NO_{11}.HCl$

Molecular weight: 579.99.

Solubility: Highly soluble in water.

pH: 4.0-5.5.

Storage: Room temperature (20°C). Photosensitive.

Mechanism of action: The cytotoxic effect of doxorubicin on malignant cells is thought to be related to nucleotide base intercalation and cell membrane lipid binding activities. The interaction of doxorubicin with the DNA double helix is considered to be an important mechanism of doxorubicin cytocidal activity. Cells treated with doxorubicin have been shown to manifest the characteristic morphologic changes associated with programmed cell death. Doxorubicin induced apoptosis may be an integral component of the cellular mechanism of action relating to therapeutic effects, toxicities, or both.

Clinical Pharmacology: The initial distribution half-life of approximately 5 minutes suggests rapid tissue uptake of doxorubicin, while its slow elimination from tissues is reflected by a terminal half life of 20 to 48 hours. Steady state distribution volume ranges from 809 to 1214 L/m2 and is indicative of extensive drug uptake into tissues. Binding of doxorubicin and its major metabolite, doxorubicinol, to plasma proteins is about 74 to 76% and is independent of plasma concentration of doxorubicin. Disposition of doxorubicinol (DOX-OL) in patients is formation rate limited, with the terminal half life of DOX-OL being similar to doxorubicin. The relative exposure of DOX-OL, i.e., the ratio between the AUC of DOX-OL and the AUC of doxorubicin, compared to doxorubicin ranges between 0.4 and 0.6. Plasma clearance is in the range 324 to 809 mL/min/m2 and is predominately by metabolism and biliary excretion. Approximately 40% of the dose appears in the bile in 5 days, while only 5 to 12% of the drug and its metabolites appear in the urine during the same time period.

References:

1. Recommentions for the safe handling of parenteral antineoplastic drugs. NIH publication No. 83-2621.
2. http://www.rxlist.com/adriarnycin-pfs-drug.htm

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4.5 Polymer profile

4.5.1 Gellan gum

Synonyms: Kelcogel®, INS no. 418.

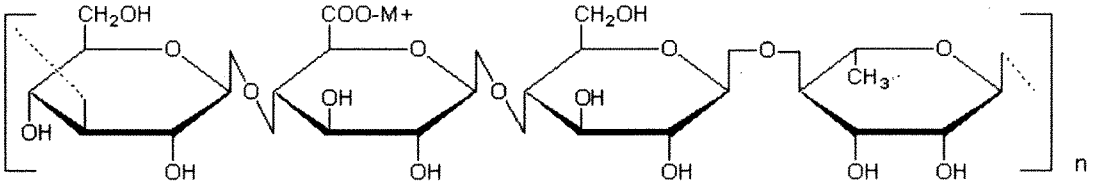
Description: Gellan gum is a high molecular weight polysaccharide gum produced by a pure culture fermentation of a carbohydrate by Pseudomonas elodea, purified by recovery with isopropyl alcohol, dried and milled.

Appearance: Off-white powder.

CAS number: [71010-52-1].

Chemical Nature: Gellan gum is a linear tetrasaccharide-4)-L-rhamnopyranosyl-(α- 1-3)-D-glucopyranosyl-(β-1-4)-D-glucuronopyranosyl-(β-1-4)-D-glucopyranosyl-(β- 1- with O(2) L-glyceryl and O(6) acetyl substituents on the 3-linked glucose. Its structure consists of four linked monosaccharides (i.e., simple sugars), including one molecule of rhamnose (a sugar found in various plants), one molecule of glucuronic acid (an oxidized glucose molecule) and two molecules of glucose (a component of sucrose, which is common sugar).

Structural formula:



Molecular weight: Approximately 500,000 dalton.

Solubility: Soluble in water, forming a slight viscous solution, insoluble in ethanol.
pH: 4.5-6.5.

Storage: In cool, dry place.

Functional use: Gellan gum is a widely accepted ingredient within the food and pharmaceutical industry and is used in many of food products. Gellan gum as gelling agent can produce textures in the final product that vary from hard, non- elastic, brittle gels to fluid gels. Its water solubility, thickening, gelling and stabilizing agent properties are some of the reason for its wide application in food and pharmaceutical industry; it is currently used in bakery fillings, confections, dairy products etc. Gellan gum is also used as an ingredient in personal and oral care applications like hair care products, creams, sunscreens etc.

Regulatory Status: It is approved for food, non-food, cosmetic and pharmaceutical use in the United States, Canada, Australia and many other countries in Latin America, South America, Asia and the European Union. According to the FDA, gellan gum may be safely used as a direct food additive for human consumption as per 21 CFR 172.665 (USA) and appears as E418 in the European Community Directive.

References: 1. Kelcogel®. http://www.cpkelco.com/food/gellan.html

2. M. O'Neill, R. Selvendran, A.J. Morris. Structure of the acidic extracellular gelling polysaccharide produced by Pseudomonas elodea. ***Carbohydr. Res.***, 1983, 124; 123-133.

4.5.2 Polyethylene glycol

Synonyms: PEG, PEG-SH, ethylene oxide polymer.

Description: Poly(ethylene glycol) is prepared by polymerization of ethylene oxide and is commercially available over a wide range of molecular weights from 300 g/mol to 10,000,000 g/mol.

Appearance: White powder.

CAS number: [25322-68-3],

Chemical Formula: HS (CH2 CH2)n OH

Molecular weight: 3000 dalton.

Structural formula:

Solubility: Soluble in water.

pH: 4.5-7.5. Storage: - 20 °C.

Functional use:

Poly(ethylene glycol) has several chemical properties that make it useful in various biological, chemical and pharmaceutical applications:

1. 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 immunogenicities.
2. Hydrophilic: easy attachment to proteins and other biomolecules, decreases aggregation and increases solubility.
3. Highly flexible: provides for surface treatment or bioconjugation without steric hindrance.

Regulatory Status: Included in the FDA inactive ingredients guide (dental preparations; i.m. and i.v. injections; ophthalmic preparations; oral capsules, solutions, syrups and tablets; rectal, topical and vaginal preparations). Included in nonparenteral medicines licensed in the United Kingdom, also included in the Canadian list of acceptable non medicinal ingredients.

The WHO has set an estimated acceptable daily intake of polyethylene glycols at up to 10 mg/kg body weight.

References:

1. FAO/WHO. Evaluation of certain food additives. Twenty-third report of the joint FAO/WHO expert committee on food additives. World Health Organ Tech Rep Ser 1980, No. 648.
2. J.M. Harris, R.B. Chess. Effect of pegylation on pharmaceuticals. **Nat. Rev. Drug Dis.**, 2003, 2; 214-221.

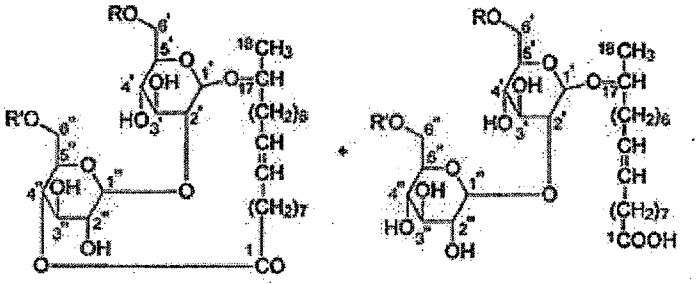
4.5.3 Sophorolipid

Synonyms: Sophorolipides, glycolipids.

Description: Sophorolipid are a kind of microbial extracellular biosurfactant produced by yeast cells. The yeast cells such as Candida bombiciola, Yarrowia lipolytica, Canada apicola and Candida bogoriensis when challenged with fatty acids (oleic acid, stearic acid etc) and glucose results in the formation of sophorolipids.

Appearance: Off-white powder.

Structure: Sophorolipid are present in the form of disaccharide sophorose a dimeric glucose.Sophorolipid can have two structures; close ring (macrolactone form) and open ring (acidic form).



Molecular weight: Sophorolipid (C_{18}) – 600

Solubility: Soluble in water

pH: 4-5

Storage : In cool, dry place.

Functional use: As a bacteriociodes in the formulation of skin and body care products.used as a surfactant ,anticancer activity.

References:

1. M. Kasture, S. Singh, P. Patel, P.A. Joy, A.A. Prabhune, C.V. Ramana, B.L.V. Prasad. Multi utility sophorolipids as nanoparticle capping agents: Syntehsis of stable and water dispersible Co nanoparticles. **Langmuir** 2007, 23; 11409-11412.

2. S. Singh, P. Patel, S. Jaiswal, A.A. Prabhune, V. Ramana, B.L.V. Prasad. A direct method for the preparation of glyoclipid metal nanoparticle conjugates: Sophorolipids as reducing and capping agents for the synthesis of water re-dispersible silver nanoparticles and their antibacterial activity. ***New J. Chem., 2009, 33; 646.652.***