FORMULATION OF *ACETYL SALICYLIC ACID* ENCAPSULATED LIPOSOMES

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Drug encapsulation

What is Encapsulation and What can it be used for?

- Inclusion of a substance inside a capsule mainly to control the release of the substance to be delivered. Encapsulation also helps to improve solubility and bioavailability of drugs.
- ‘Controlled release’ strategies are highly prized in medicine since they can allow drugs to be absorbed more slowly, at a specific location in the body or at the say-so of an external trigger.

Examples of nano and microcapsule designs for selected release mechanisms:

Slow release - release payload slowly over a longer period of time

Quick-release – break open upon contact with a surface (e.g. when pesticide hits a leaf)

Specific release - breaks open when a molecular receptor binds to a specific chemical (e.g., upon encountering a tumour or protein in the body)

Moisture release - release contents in the presence of water (e.g. in soil)

Heat-release – release on warming above a certain temperature

pH release - nanocapsule breaks up only in specific acid or alkaline environment (e.g., in the stomach or inside a cell)

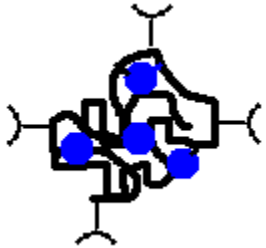
Ultrasound release - the capsule is ruptured by an external ultrasound frequency

Magnetic release - a magnetic particle in the capsule ruptures the shell when exposed to a magnetic field

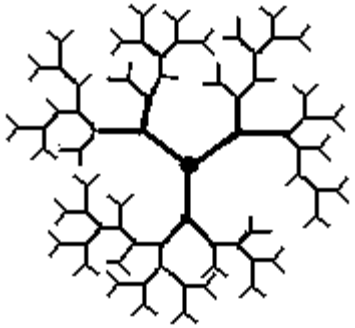
DNA nanocapsule – release foreign DNA into cells to express specific proteins (used for DNA vaccines)

Matrices for drug encapsulation

Polymer-drug conjugate

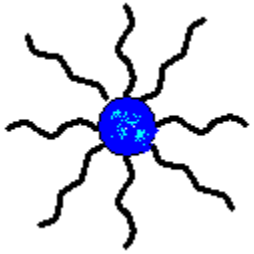


Polymer- drug-targeting ligand conjugate



Dendrimer

Polymeric NanoParticle with attached ligands

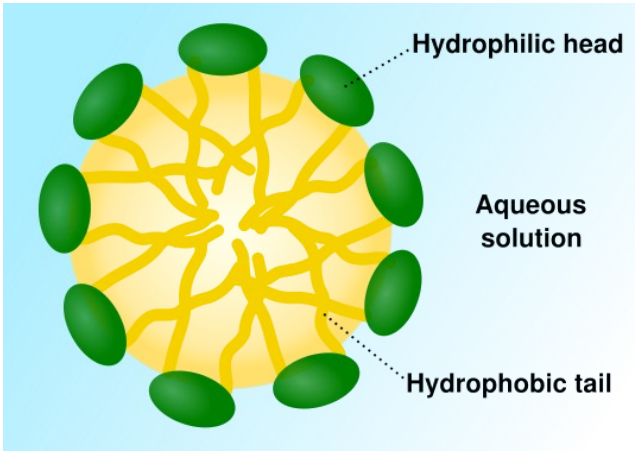


Polymer micelle

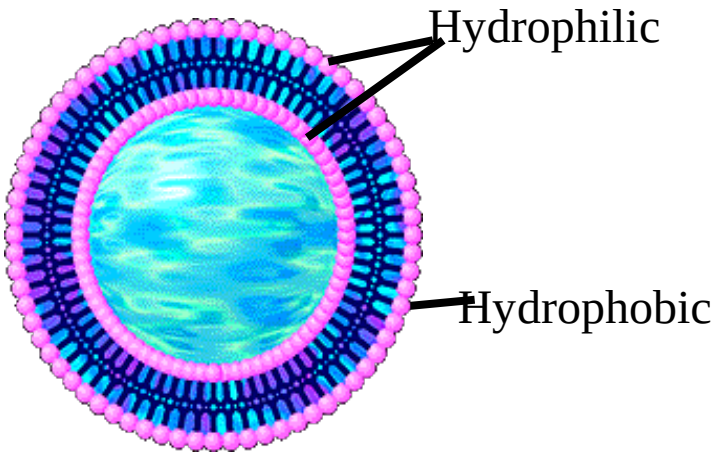
- Polymer matrices for encapsulation

Matrices for drug encapsulation

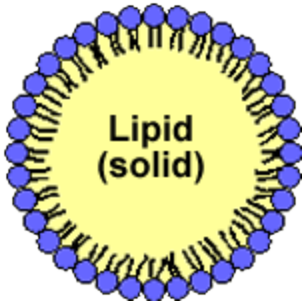
Micelle

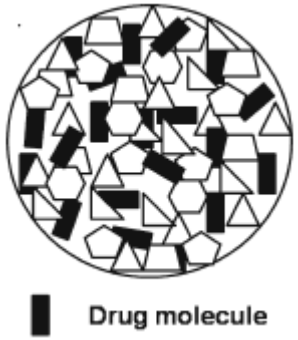


Liposome



Solid Lipid Nanoparticle





Nanostructured lipid

- Lipid matrices for encapsulation

Why use liposomes in drug delivery?

- Reformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices (TI) of various agents mainly through alteration in their bio distribution.

The therapeutic applications of liposomes

1.Formulation aid

Liposomes are made up of lipids which are relatively non-toxic, non-immunogenic, biocompatible and biodegradable molecules, and can encapsulate a broad range of water-insoluble (lipophilic) drugs

2.Site-avoidance delivery

Liposomes are taken up poorly by tissues such as heart, kidney, and GI tract, which are major sites for toxic side-effects of a variety of anti neoplastic drugs.

3. Site-specific delivery

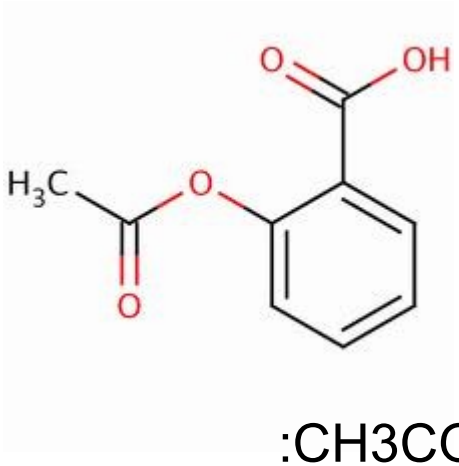
Delivery larger fraction of drug to the target site and therefore, reducing exposure to normal tissues

4. Release

OBJECTIVES

- Synthesize nanoparticulate liposomes using liquid crystals and egg yolk lecithin
- Encapsulate partially water soluble drug- Acetyl Salicylic Acid
- Study drug release for possible slow release/pH release

Acetylsalicylic Acid (Aspirin)

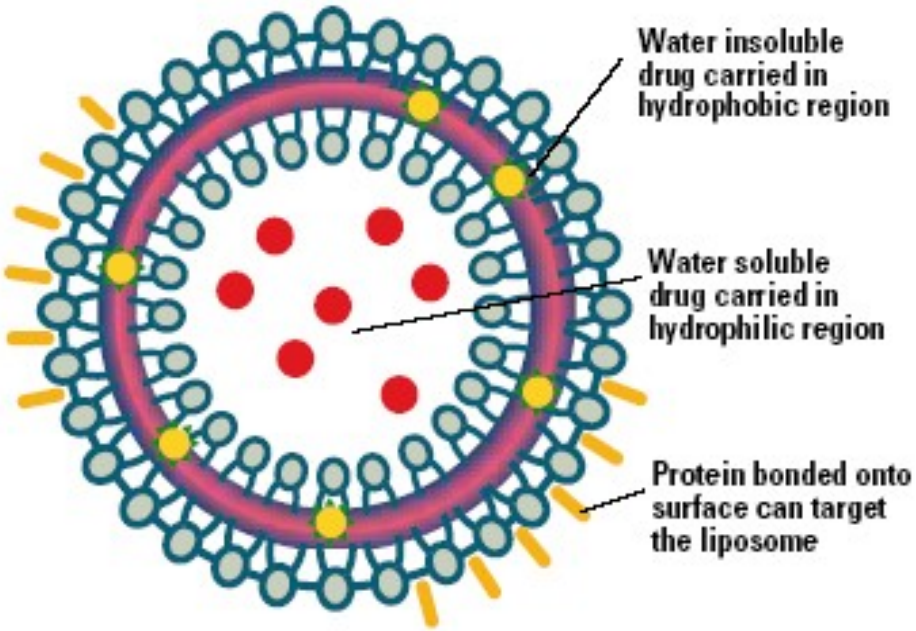


Formula :CH3COOC6H4COOH
Molecular wt. :180.16
Toxicity Oral rat :LD50: 200 mg/kg
Physical properties : White, crystalline, weakly acidic substance, melting point 137°C, partially water soluble
Uses

- As a relief of headache and muscle and joint aches.
- Reducing fever, inflammation, and swelling and thus has been used for treatment of rheumatoid arthritis, rheumatic fever, and mild infection.

Encapsulation in liposomes

Thin Film Hydration Technique



- Entrap agents which are virtually insoluble in water and can be incorporated into the lipid bilayer during vesicle formation.

These agents are generally treated as lipids and are mixed homogeneously with the lipid component prior to vesicle hydration step

Preparation of Liposomes

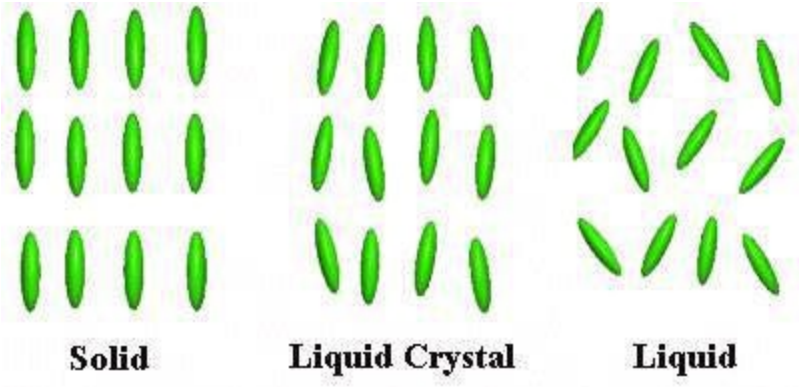
- Egg yolk is a good source of phospholipids (PL)

  The main components of egg yolk Lecithin are Phosphatidylcholine (PC,80.5%, true lecithin from the chemical point of view) and Phosphatidylethanolamine (PE,11.7%)

- The carbohydrate liquid crystal used was β-sitosteryl 2,3,4,6,-tetra-O-acetyl-β-D-glucopyranoside

Carbohydrate liquid crystals for preparation of liposomes

- Introduction
  - Liquid crystal is the fourth state of matter that has properties of both the liquid and solid states
  - A liquid crystal may flow like a liquid, but has the molecules in a liquid arranged and/or oriented in a crystal like way



Carbohydrate Liquid Crystals

- Carbohydrate liquid crystals are usually composed of monosaccharide derivatives with one or more alkyl chains linked via (thio)-ether, ester, or amide linkages.
- Amphiphilic carbohydrates have been used as tools for molecular recognition in organized system

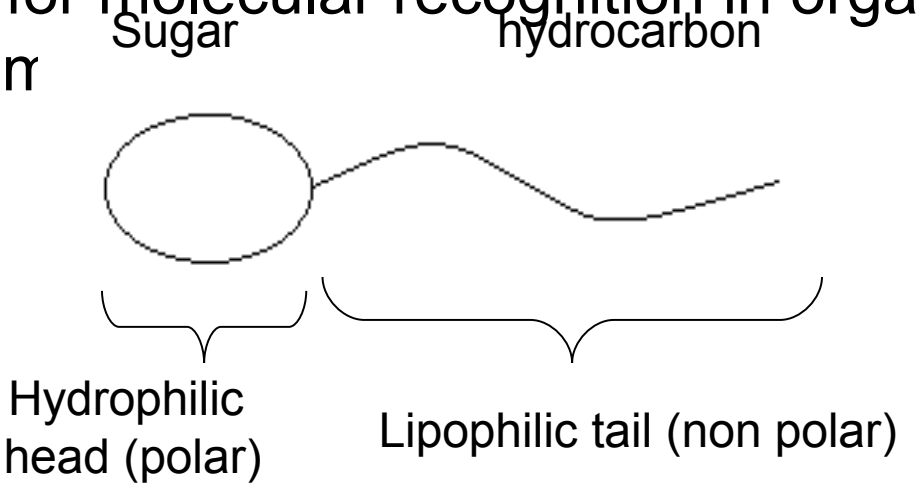
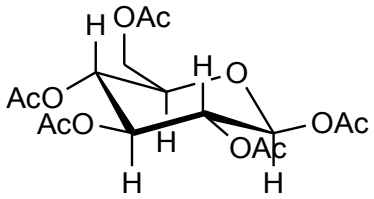


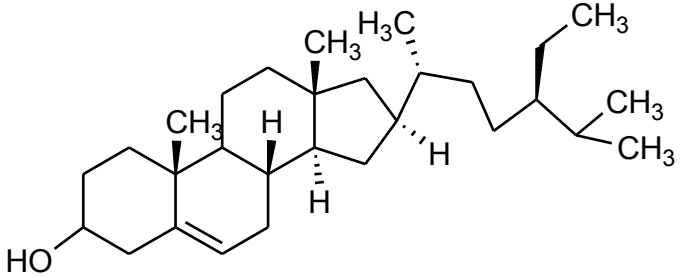
Figure : Structure of an amphiphilie

SYNTHESIS OF GLYCOLIPIDS

General principle:

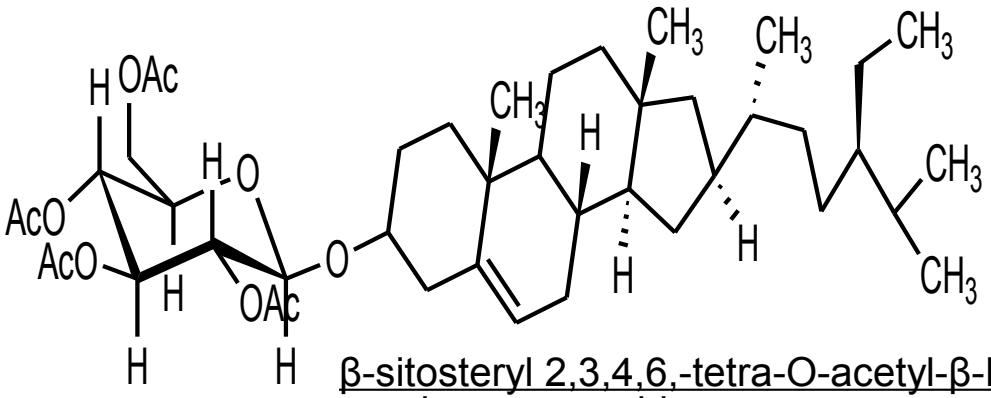
Glycoside bond formation reaction between aglycone and an activated anomeric center





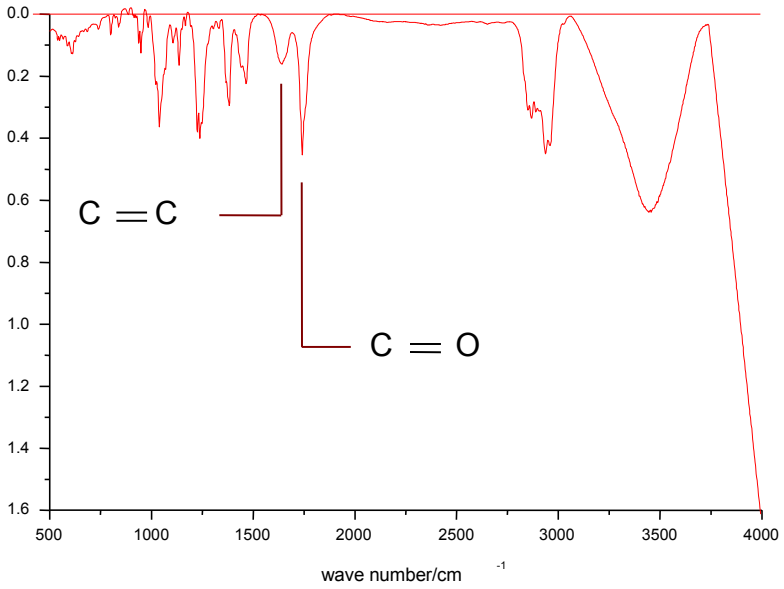
$BF_3.Et_2O/CH_2Cl_2$

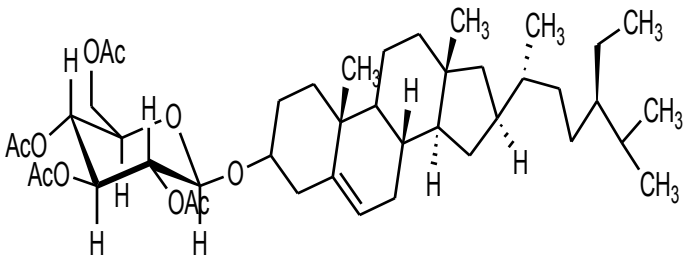
GLYCOSIDE BOND



β-sitosteryl 2,3,4,6,-tetra-O-acetyl-β-D-glucopyranoside

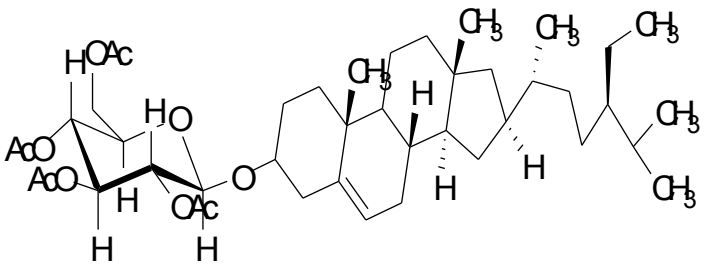
Characterization of β-sitosteryl 2,3,4,6,-tetra-O-acetyl-β-D-glucopyranoside

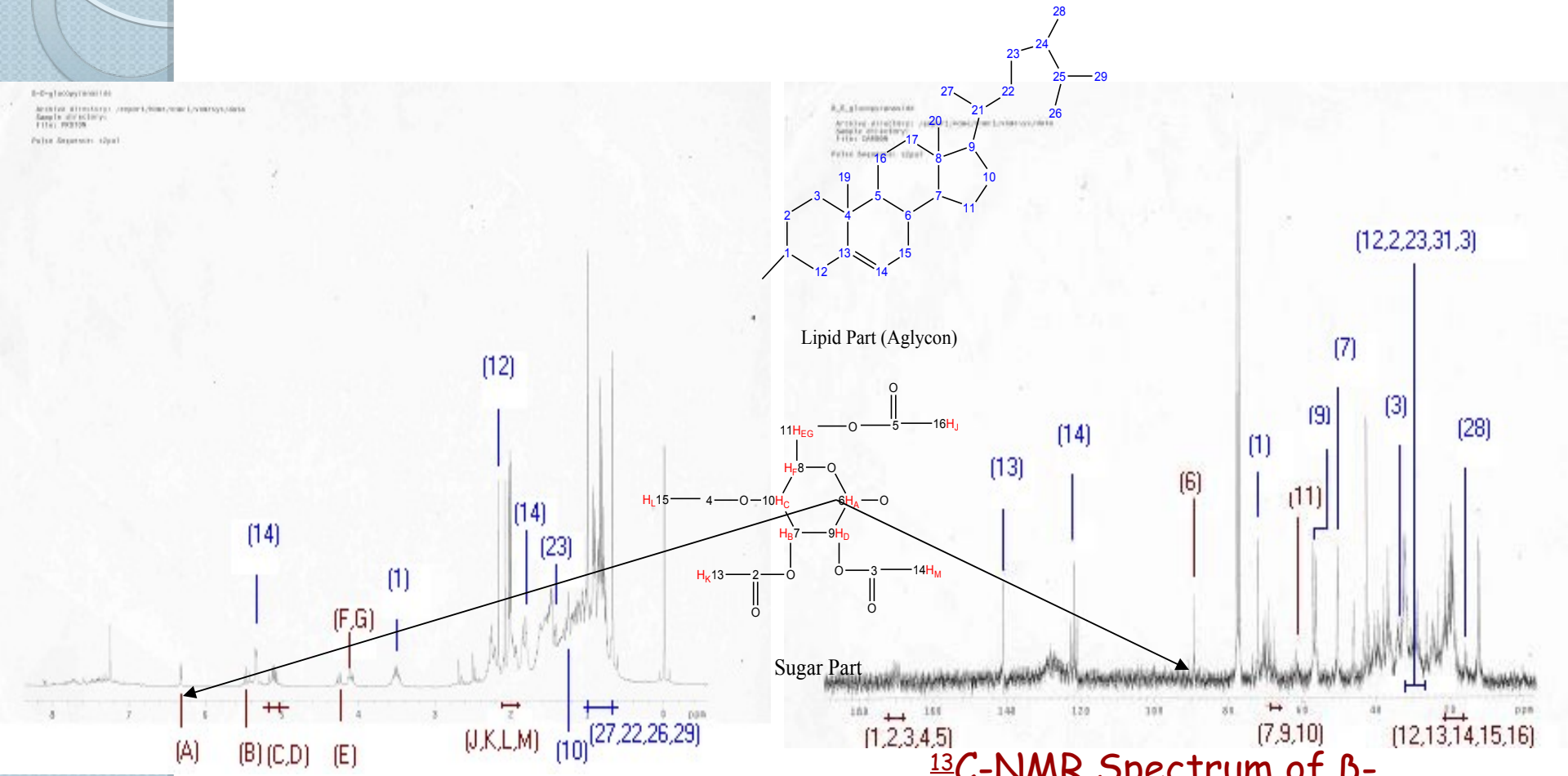




FT-IR Spectrum of β-sitosteryl 2,3,4,6-tetra-O-acetylglucopyranoside

NMR Spectroscopic assignments:



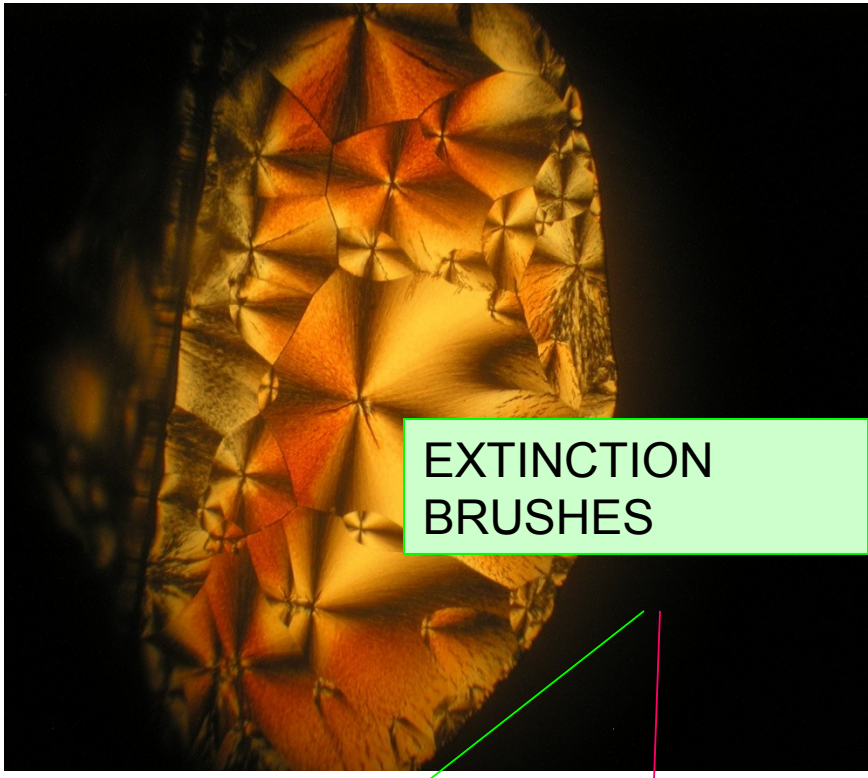


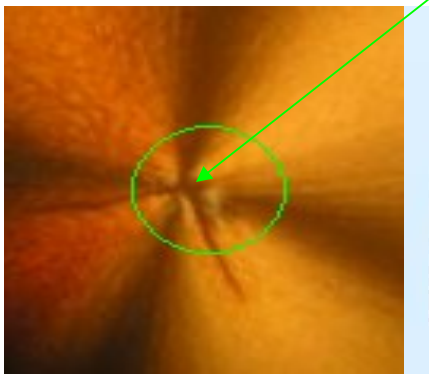
[1]H-NMR Specrtum of β-Sitosteryl 2,3,4,6-tetra-O-acetylglucopyranoside

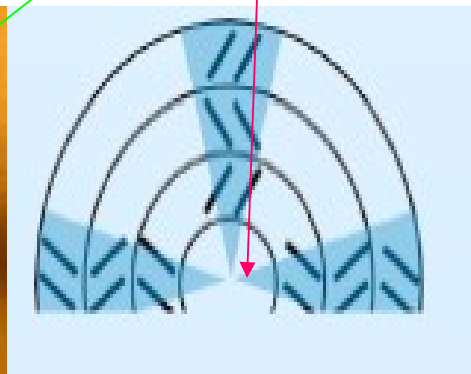
[13]C-NMR Spectrum of β-Sitosteryl 2,3,4,6-tetra-O-acetylglucopyranoside

POLARIZABLE MICROSCOPY

•Thermotropic liquid crystalline properties were shown by β-Sitosteryl 2,3,4,6-tetra-O-acetyl glucopyranoside.



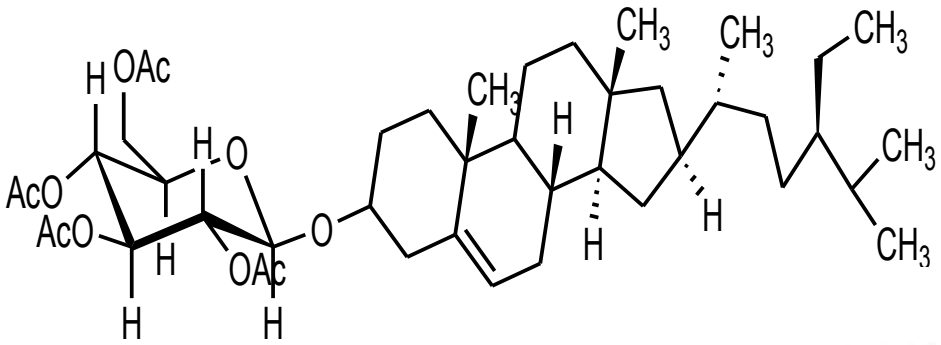


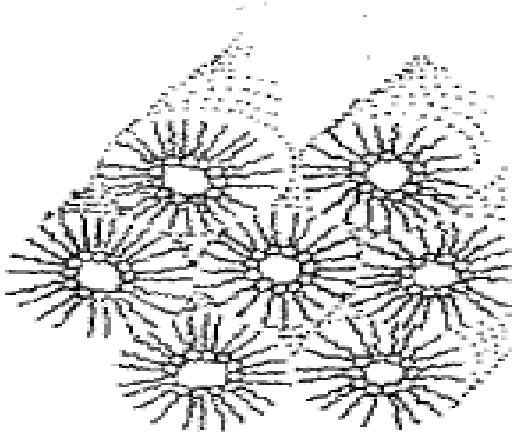


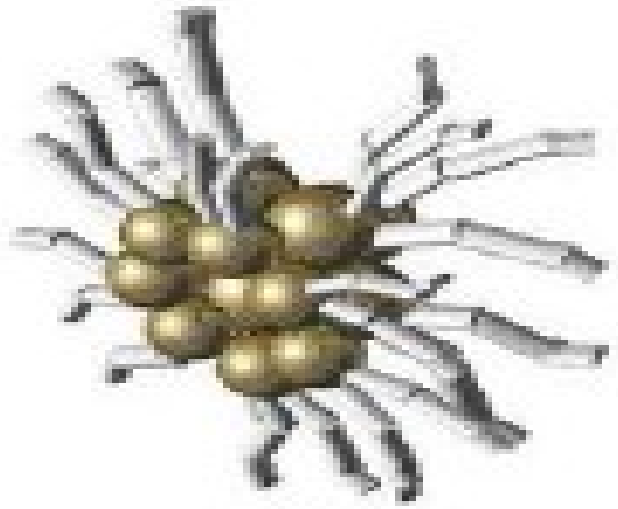
According to the literature,

- The texture observed was characteristic for the hexagonal columnar phase due to the presence of extinction brushes.

A cylindrical arrangement with sugars surrounded by alkyl/steroidal groups.







The cylinders are arranged in the best way of packing which is hexagonal lattice.

Preparation of Acetyl salicylic acid Encapsulated Liposomes

Compositions taken for the preparation of encapsulated liposomes

Sample No	Amount of PL/mg	Amount of LC/ mg	Total amount /mg	Amount of Aspirin/mg
1	10.0	-	10	1.5
2	7.5	2.5	10	1.5
3	5.0	5.0	10	1.5
4	2.5	7.5	10	1.5
5	-	10.0	10	1.5

Encapsulation efficiency

Procedure:

- The suspensions centrifuged at 10000 rpm for 20 min at 4 ^{o}C
- The supernatants were collected quantitatively and filtered through 0.45μm membrane filters
- Measured UV- Absorbance of supernatants (275-280 nm)

Encapsulation Efficiency %

$$= \frac{\text{Total aspirin-Free aspirin (in supernatant)}}{\text{Total aspirin}} \times 100$$

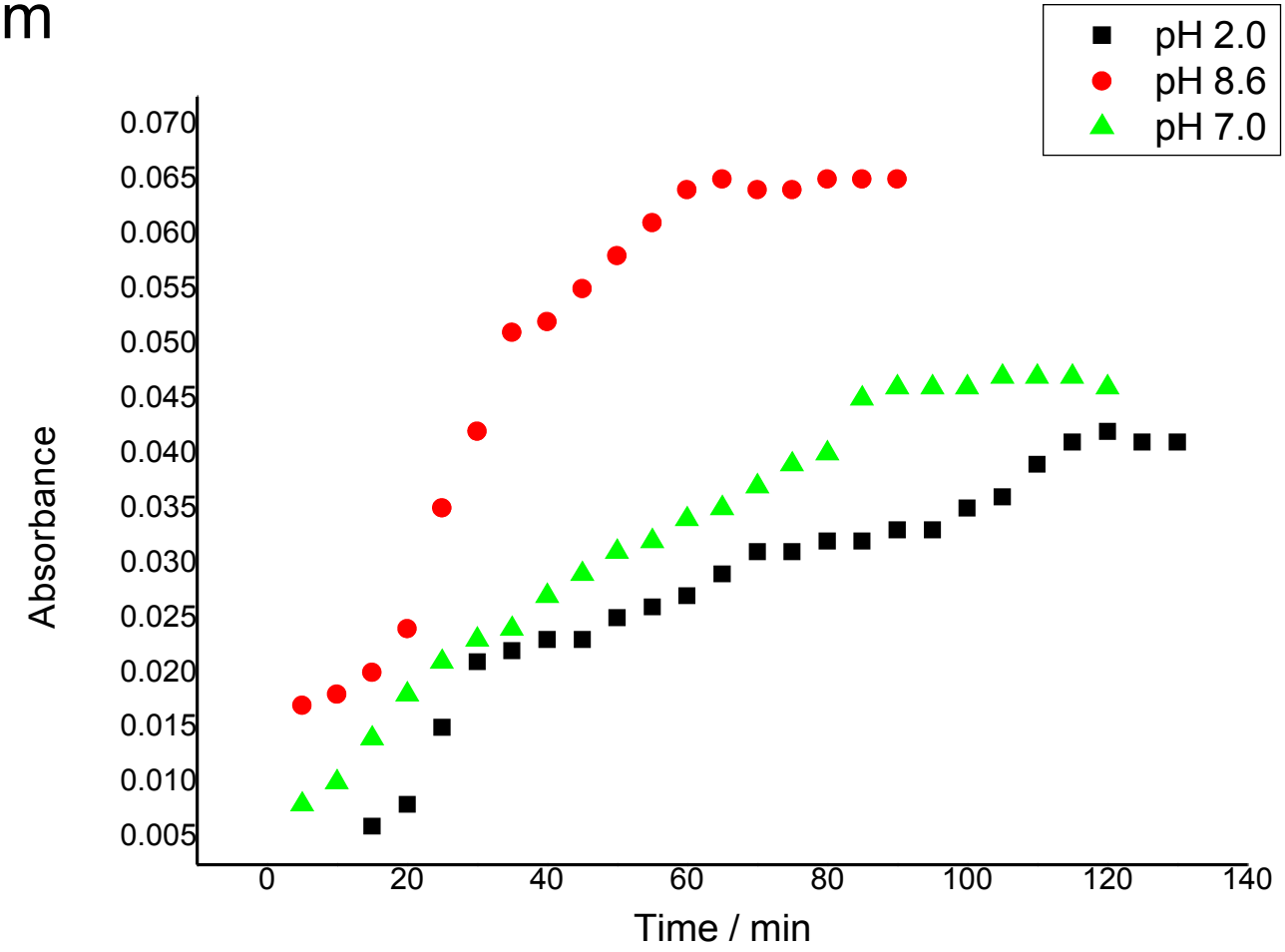
Encapsulation efficiency

Absorbance of total aspirin (150 ppm) added = 1.046

Sample No	Absorbance (supernatant)	Concentration (supernatant) /ppm	Encapsulation efficiency %
1	0.287	41.2	72.5
2	0.222	31.8	78.8
3	0.174	24.9	83.4
4	0.205	29.4	80.4
5	0.256	36.7	75.5

Drug release

Release of aspirin from liposomes (sample 3) was conducted by dialysis in a dialysis sac with 50.00 ml of deionised water and buffer solutions as the dialyzing medium



Conclusion

- Liposomes with 50% PL and 50% LC. (sample 3) has the highest encapsulation efficiency.
- Rapid drug release (burst release) was obtained at pH 8.6 and slow release observed at pH 2.0.
- This system would be able to prevent stomach irritation and unload its active ingredients in the intestine where the drug could be taken up.

References

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- Liangfang Zhang ,Steve Granick, How to Stabilize Phospholipid Liposomes (Using Nanoparticles), Materials Research Laboratory and Department of Chemical & Biomolecular Engineering, University of Illinois, Urbana, Illinois 61801.
- Luz E. Palacios , Tong Wang, Egg yolk lecithin fractionation and characterization, Department of Food Science and Human Nutrition Center for Crops Utilization Research,State University, Iowa, 50011-1061.

Acknowledgement

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THANK YOU

Where nature finishes producing its shapes, there man begins, with natural things and with the help of nature itself, to create infinite varieties of shapes.

Leonardo Da Vinci