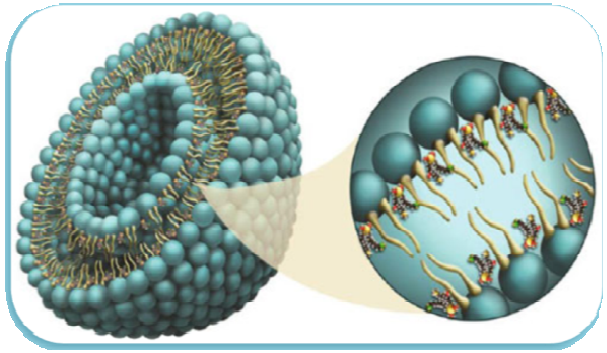
Assessment of biological activity of Chios mastic gum (acid, neutral and total fraction) extracts before and after encapsulation in liposomes.

O. Gortzi, D.-A. Karagkini, D. Kouretas, J. Tsaknis



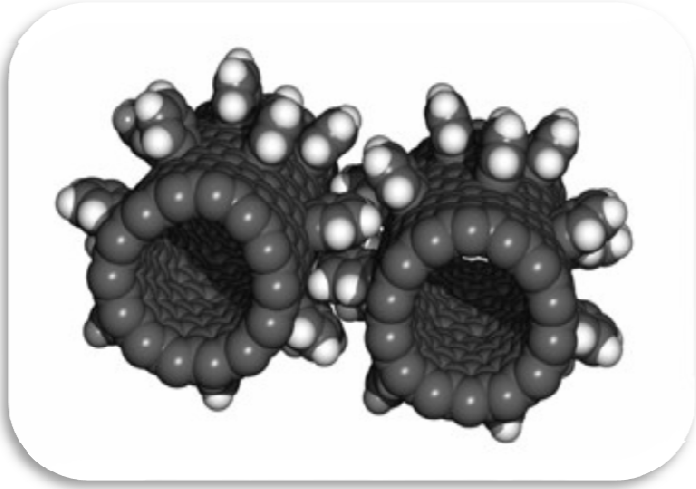




- Mastic is a well-known natural resin from the trunk and branches, of Pistacia lentiscus var. chia (Anacardiaceae), which is grown as endemic only in the Greek island of Chios.
- It has been used in traditional Greek medicine for various gastrointestinal disorders, while the plant has been mentioned by famous ancient Greek physicians (Dioscorides, Theophrastos, etc.) recommending its healing properties.

What is Nanotechnology?

Nanotechnology is the study of nanoparticles, and by definition, a nanoparticle is any material measuring less than 100 nanometers in at least one dimension.

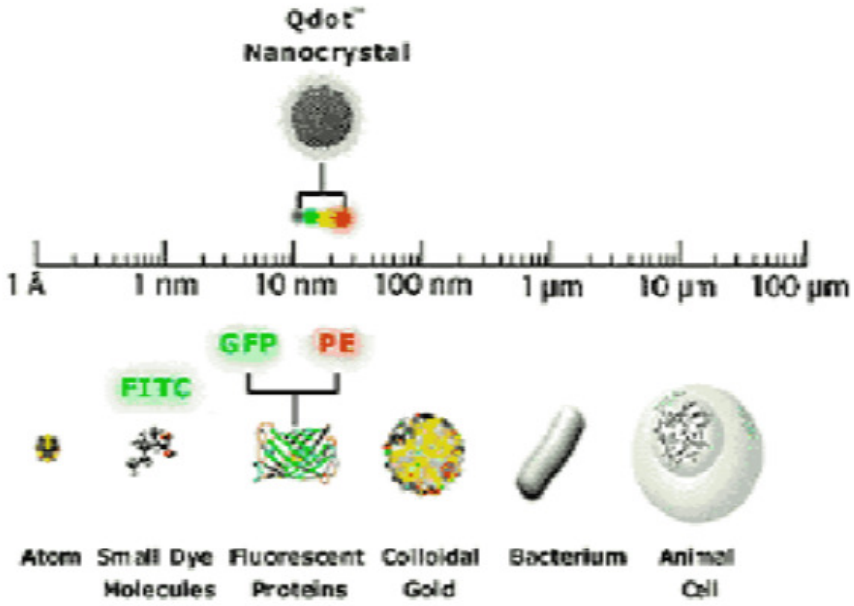




Nanomaterials

Nanomaterials may be sophisticated structures or devices that:

- ✓ are created ("nanoengineered") to perform specialized functions,
- ✓ or they may simply be smaller versions of larger materials ("nanoscale").



What are Liposomes

- Liposomes are composite structures made of phospholipids and may contain small amounts of other molecules.
- Structurally , liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid'
- Bilayer mainly composed of natural or synthetic phospholipids.

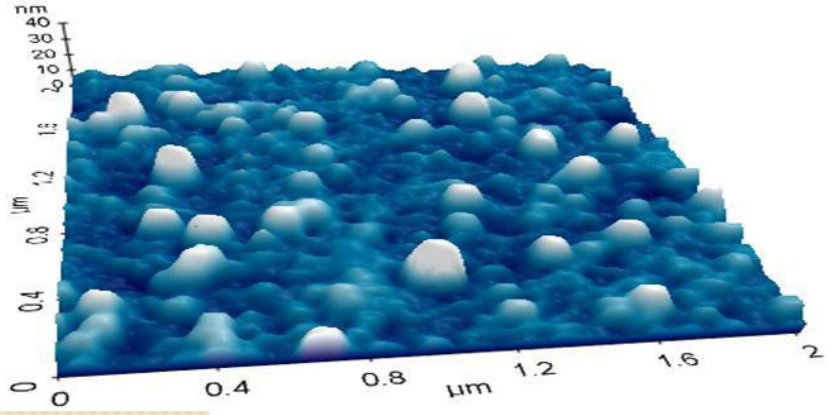
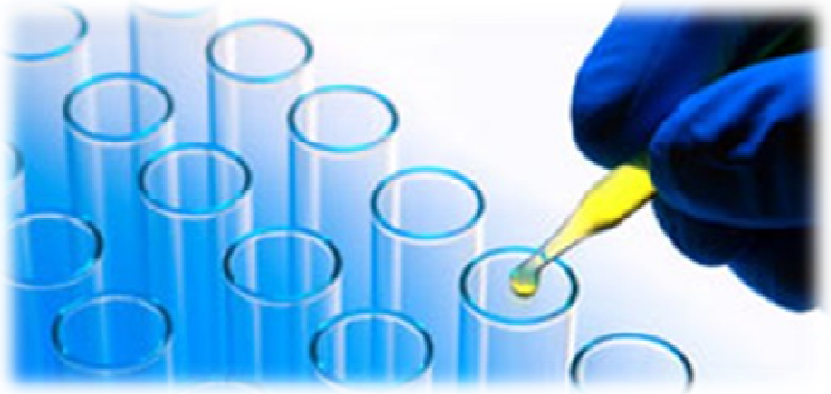


Figure 1. 3D rendering of AFM images of lliposomes in liquid with the XE-100 (2 µm size scan)

Why to use Liposome??

Basic reasons for using liposomes as bioactive components carriers:

- Direction
- Duration
- Protection
- Internalization
- Amplification

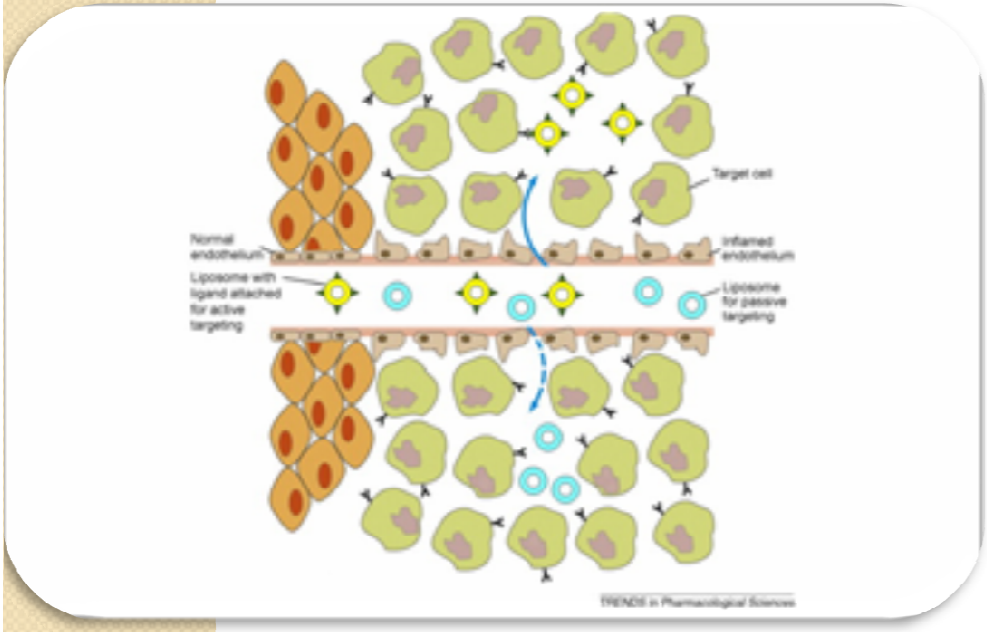


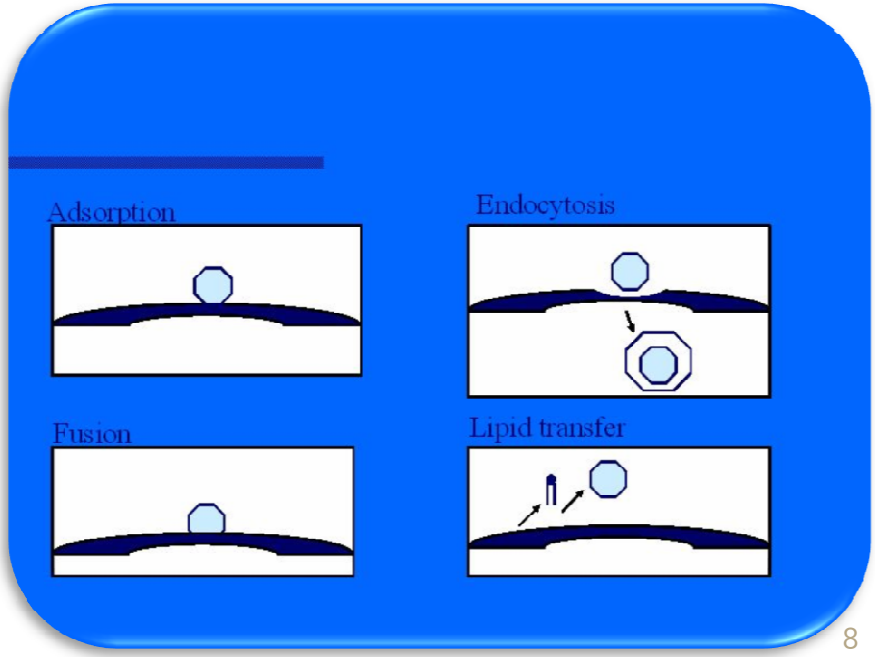
Advantages of liposomes

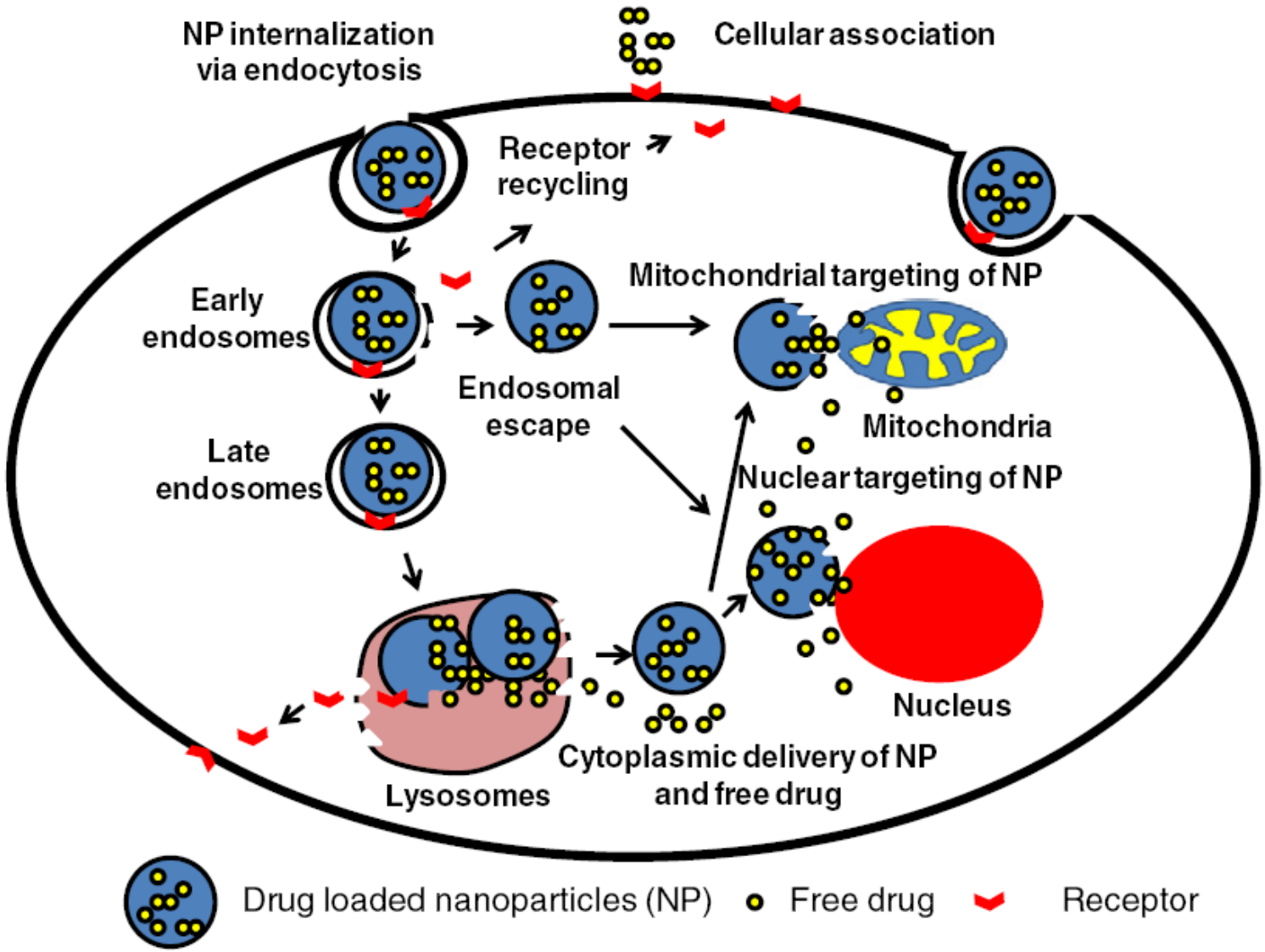
- Direction. Liposomes can target to the in-tended site of action in the body, thus enhancing its therapeutic efficacy (drug targeting, site-specific delivery).
- Duration. Liposomes can act as a depot from which the entrapped compound is slowly released over time.
- Protection. Bioactive molecules incorporated in liposomes, in particular those entrapped in the aqueous interior are protected against the action of detrimental factors.

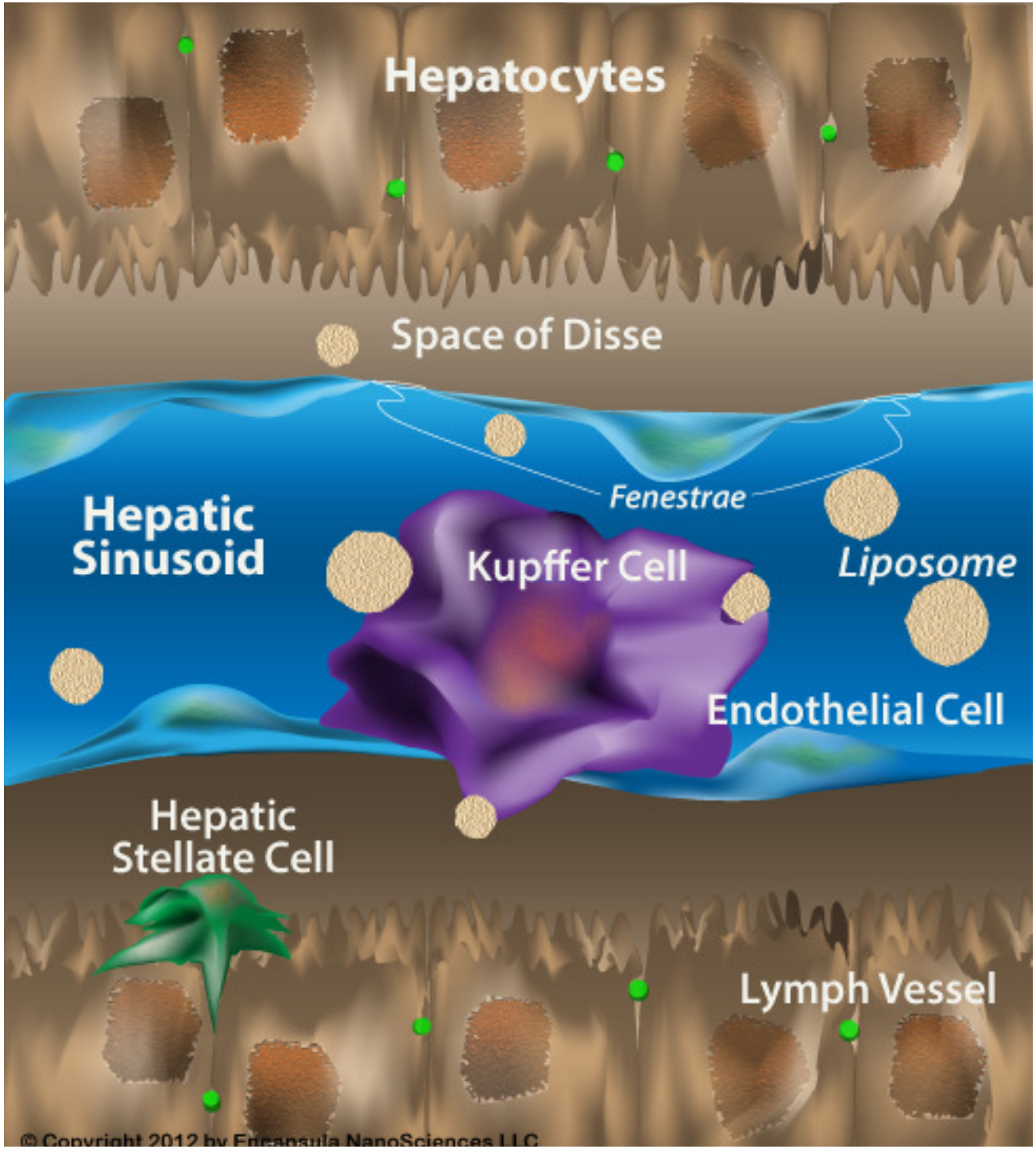
Advantages of liposomes (cont)

- Internalization. Liposomes can interact with target cells in various ways
- Are therefore able to promote the intracellular delivery of bioactive molecules molecules that in their 'free' form (i.e. non-encapsulated) would not be able to enter the cellular interior.






NP internalization
via endocytosis
Cellular association
Receptor
recycling
Early
endosomes
Mitochondrial targeting of NP
Endosomal
escape
Mitochondria
Late
endosomes
Nuclear targeting of NP
Nucleus
Lysosomes
Cytoplasmic delivery of NP
and free drug
Drug loaded nanoparticles (NP)
Free drug
Receptor


Hepatocytes
Space of Disse
Fenestrae
Hepatic Sinusoid
Kupffer Cell
Liposome
Endothelial Cell
Hepatic Stellate Cell
Lymph Vessel
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Pistacia lentiscus var. chia

- *Chios mastic gum, the resin obtained as an exudate from the trunk and branches of Pistacia lentiscus L var. chia, has found extensive use in pharmaceutical products and as a nutritional supplement.*
- *A total mastic extract without polymer was prepared after removal of the contained insoluble polymer of poly-β-myrcene in order to ameliorate solubility and enhance in vivo activity.*
- *To overcome the drawbacks of mastic gum extracts (acidic and a neutral fraction), the selection of a suitable carrier is crucial.*

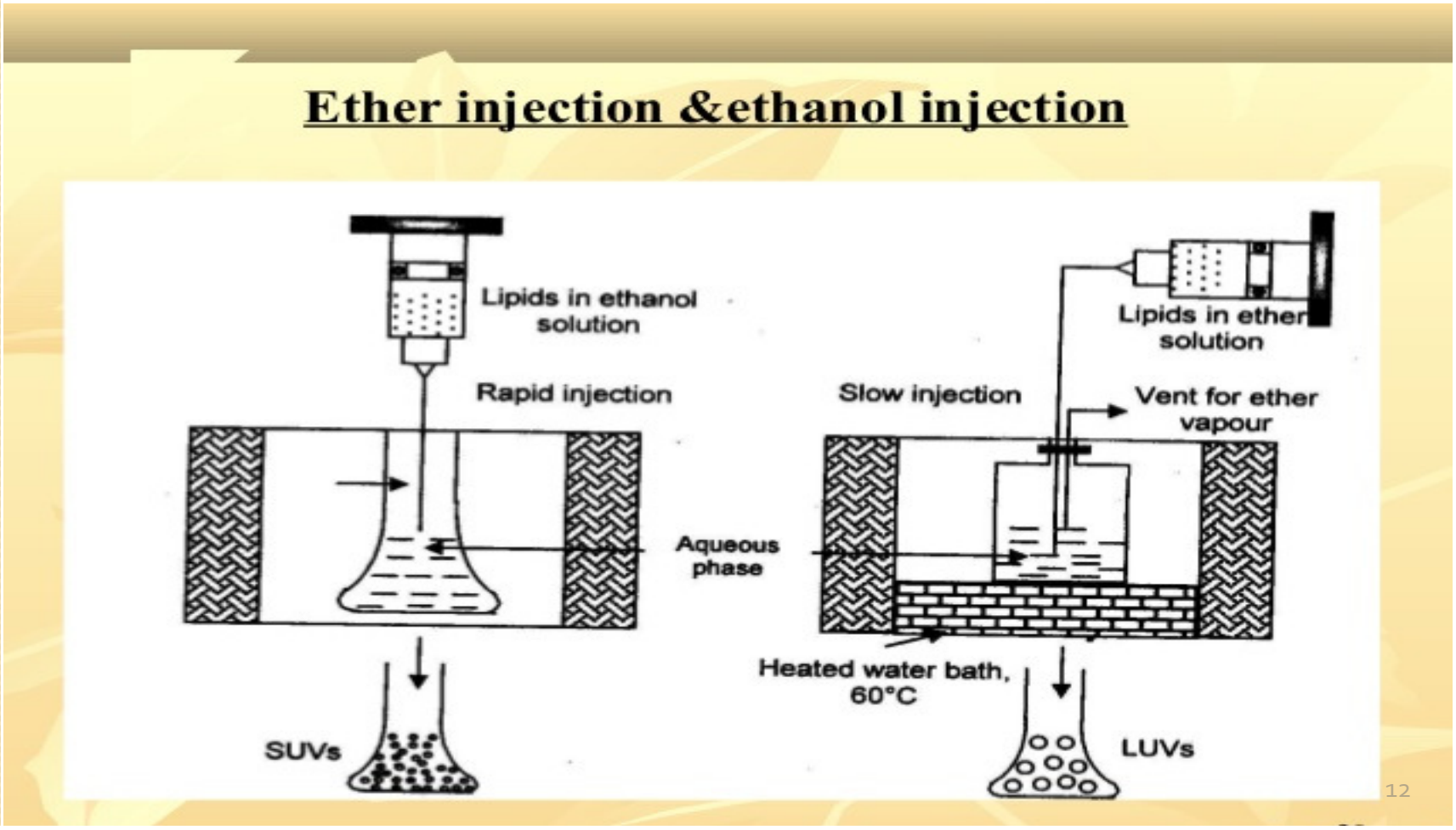






Method of liposome preparation:

Ethanol Injection (EI)



Total mastic gum extract/liposomes interaction was studied using:

- **Fourier transform infrared spectroscopy (FT-IR) and differential scanning calorimetry (DSC).**
- **The effects of different preparation methods on the physicochemical properties of colloidal systems were evaluated by:**

- Means of (SEM),
- Size distribution using a particle size analyser.

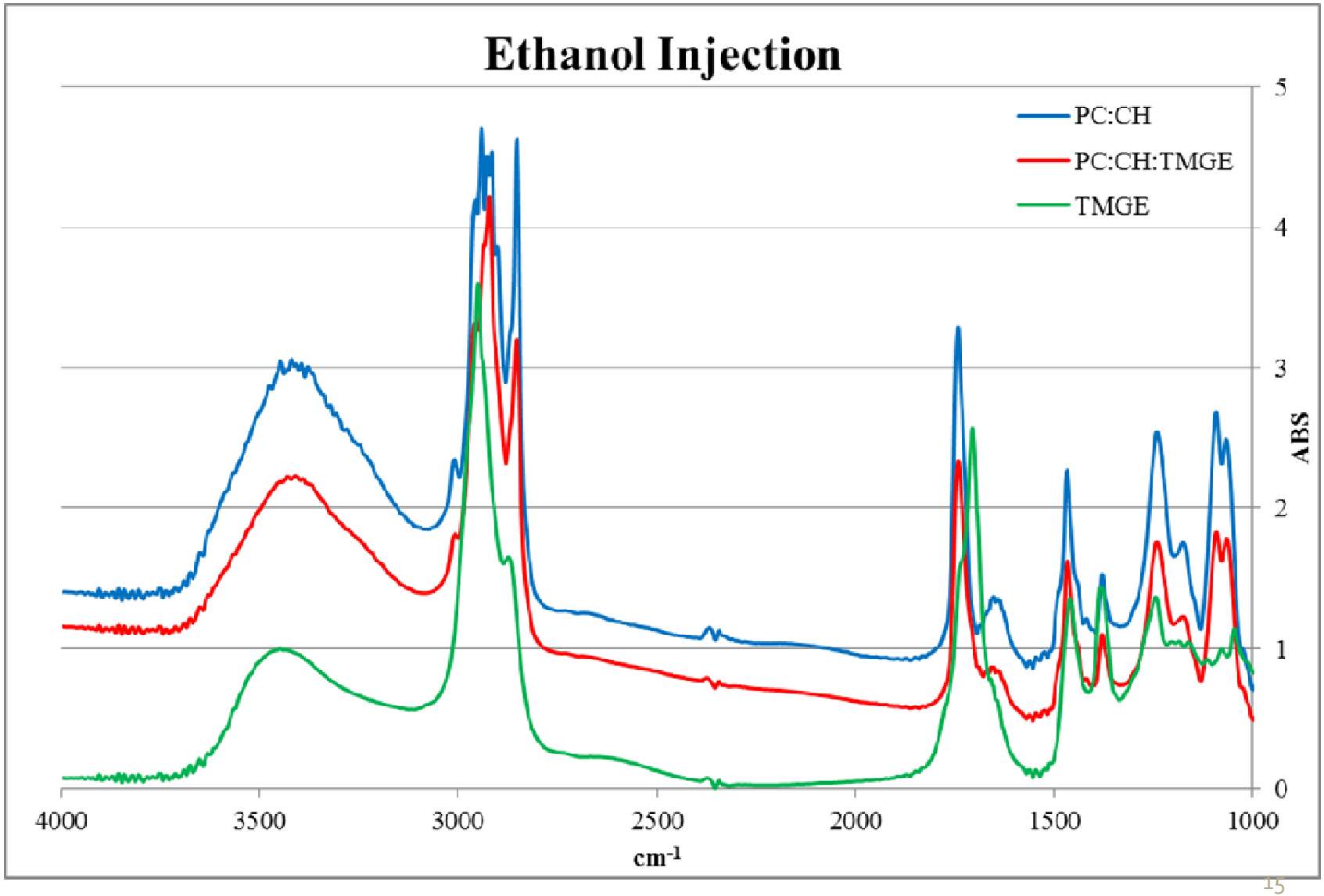
Particle size distribution

The mean particle size and polydispersity of liposomes obtained with EI method and loaded with TMGE, NMGE, AMGE are summarized in Table 1.

Extract used	Mean particle size (nm)	Polydispersity
TMGE	110.3±2.5	0.208±0.003
NMGE	115.9±3.8	0.356±0.004
AMGE	118±2.3	0.323±0.006

- ***Table 1particle size distribution of liposomes.***

Comparative FT-IR graphs of EI preparations

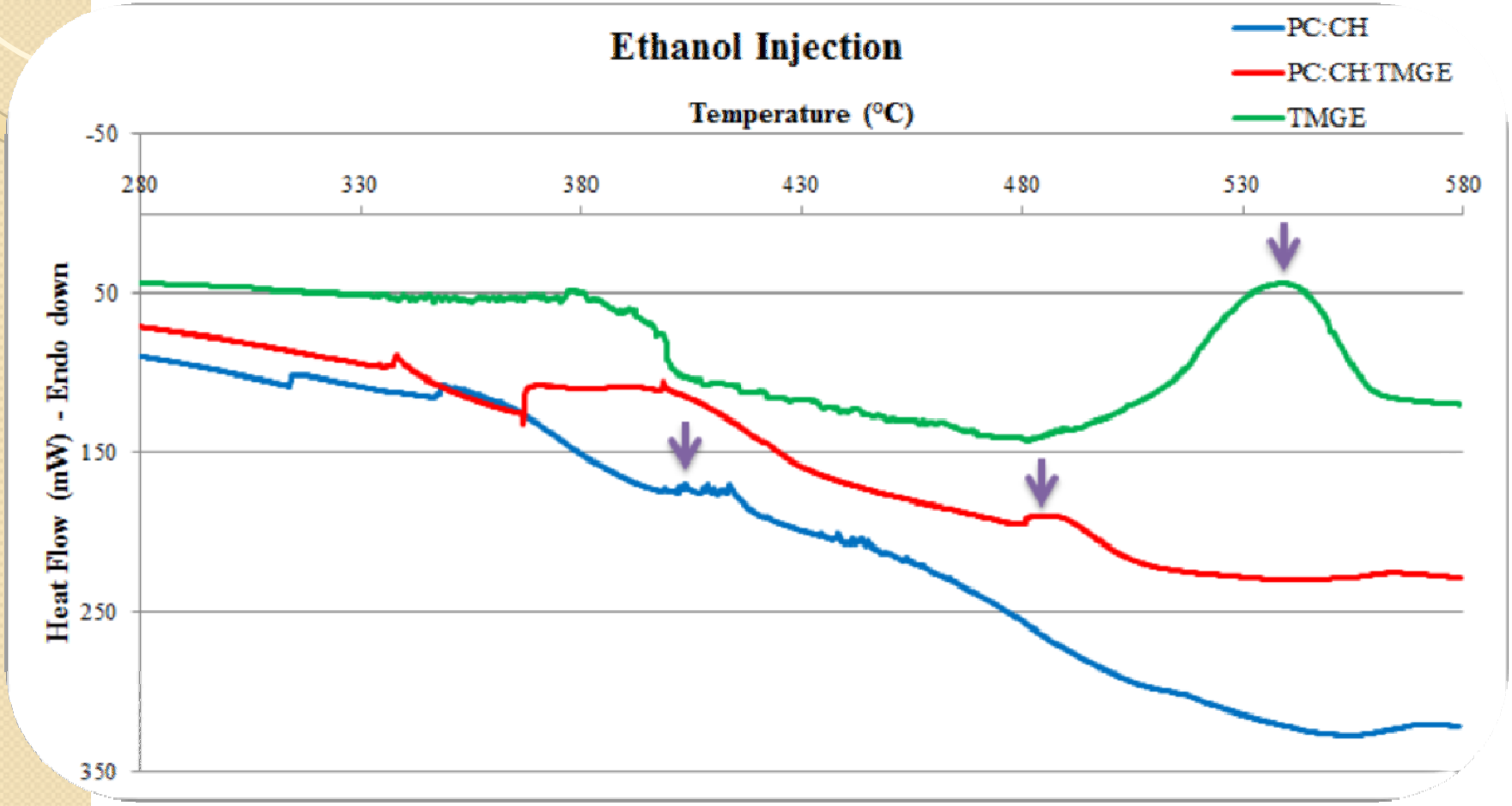


FT-IR interpretation of EI preparations (empty and TMGE loaded).

Functional Group Names	Type of Vibration	Empty TFE	TFE TMGE loaded	Empty EI	EI TMGE loaded	TMGE
Alcohol	**O-H** stretch	**3444.87**	**3446.79**	3448.72	**3431.36**	3448.72
Ester	**C=O stretch**	1741.72	**1739.79**	1741.72	1741.72	**1705.07**
Phosphate ester	**P=O** stretch	1240.23	1240.23	1240.23	1240.23	**1242.16**

- *Spectra in the transmission mode were carried out at the region of 4000-400 cm^{-1}. In the case of the stretching vibration of* ***OH groups (3600-3000 cm^{-1})*** *hydrogen bonds between TMGE components and the containing phospholipids were proved.*
- *In the case of the stretching vibration* ***of C=O of ester groups (1750-1735 cm^{-1})*** *hydrogen bonding to the ester carbonyl groups in the case of TMGE loaded liposomes were take place.*
- *The stretching vibration related to* ***the P=O bond (1720-1236 cm^{-1})*** *assumed the interaction between the polar heads of phospholipids and TMGE compounds.*

Thermal profile of EI preparation with DSC.

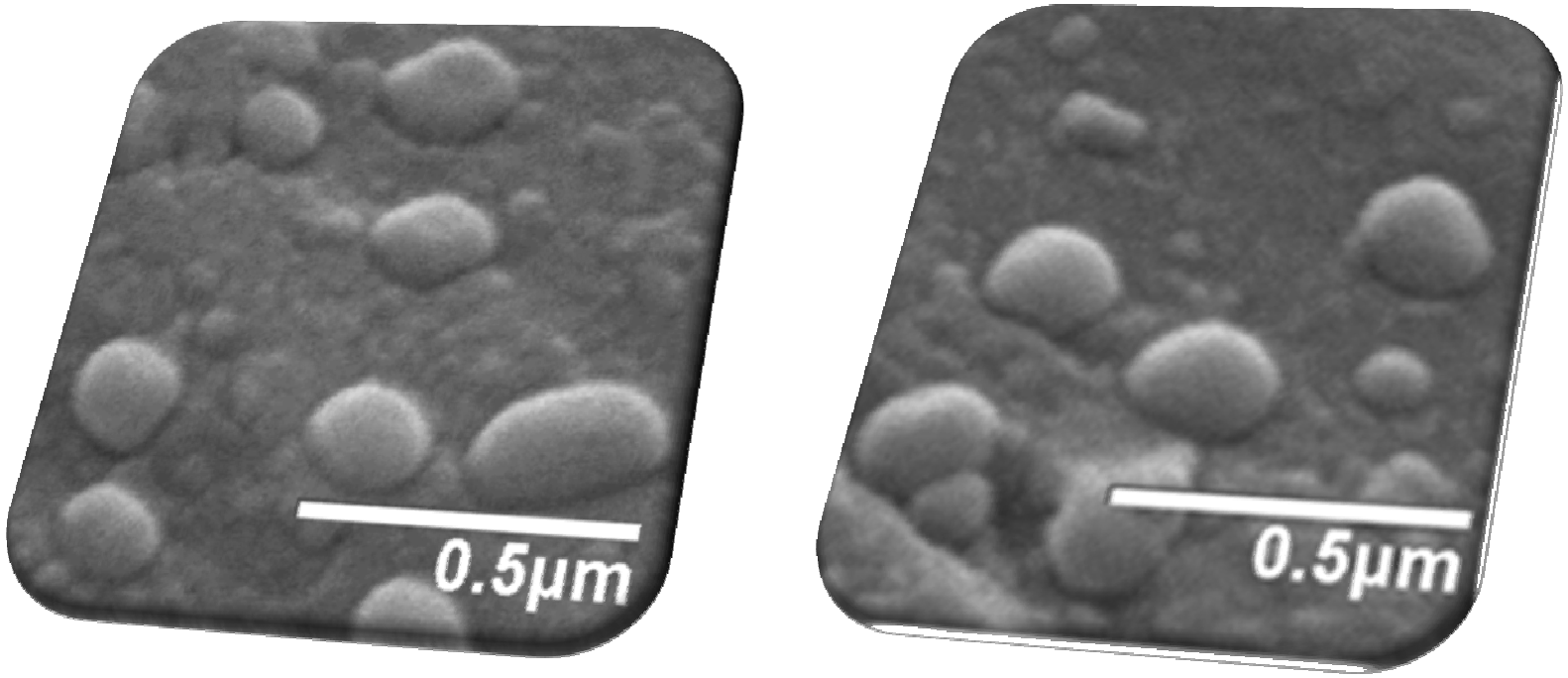


- ***The oxidative stability of prepared liposomes increased after encapsulation .***
- ***The encapsulated TMGE possess new physicochemical characteristics due to possible interactions between the compounds of TMGE extracts and phospholipids.***

- Thermal oxidative decomposition EI preparations (empty and TMGE loaded liposomes) was studied by the DSC method, using the onset temperature (T_o) of curves at the point where the auto-oxidation process begins.

- The encapsulated TMGE possess new physicochemical characteristics due to possible interactions between the compounds of TMGE extracts and phospholipids.

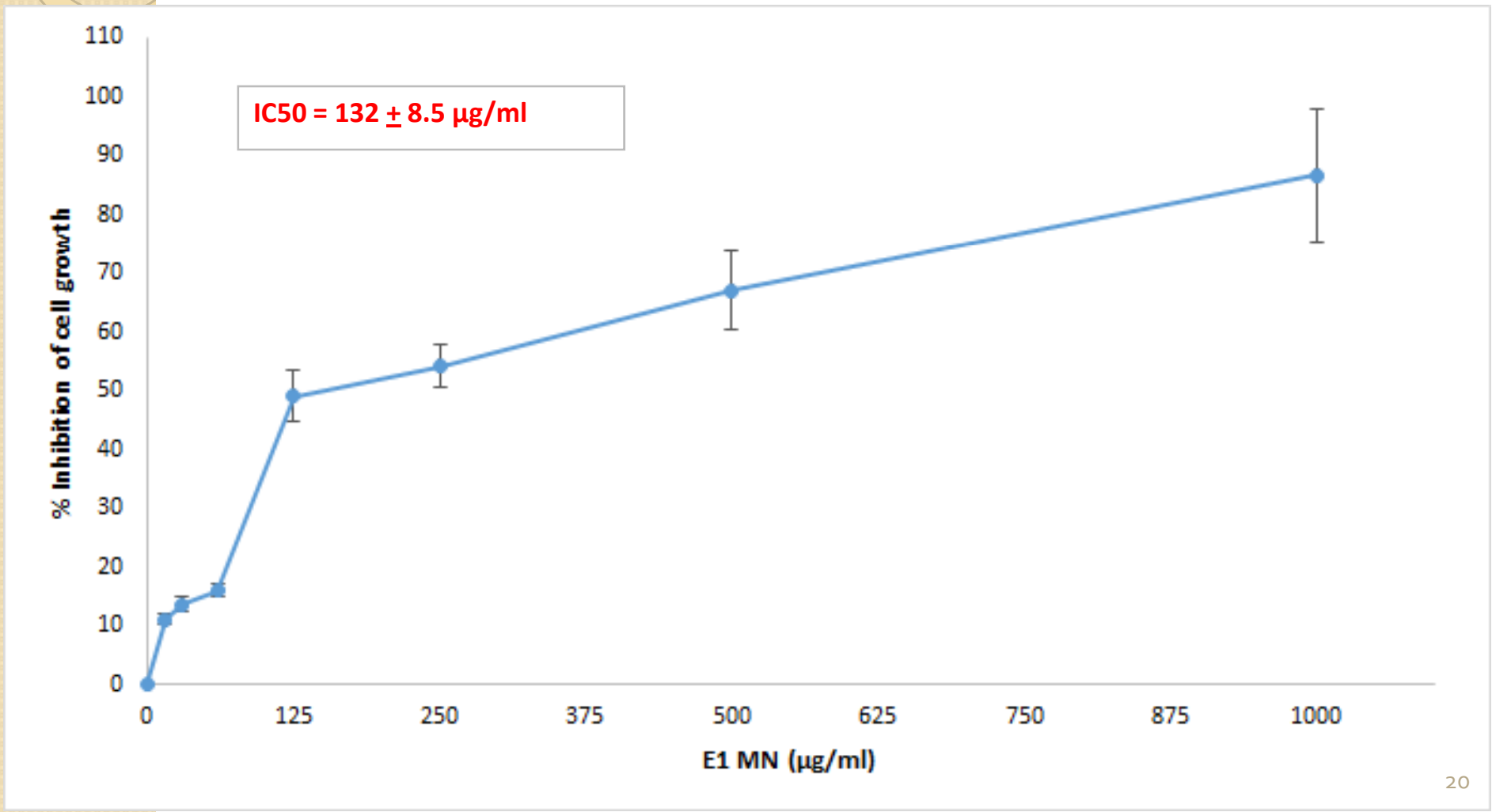
Scanning electron microscopy



- *The SEM photograph of optimized formulation revealed that particles were roughly spherical. Additionally, an uniformity was observed.*
- *An average particle size below 120 nm could be achieved and reproduced.*

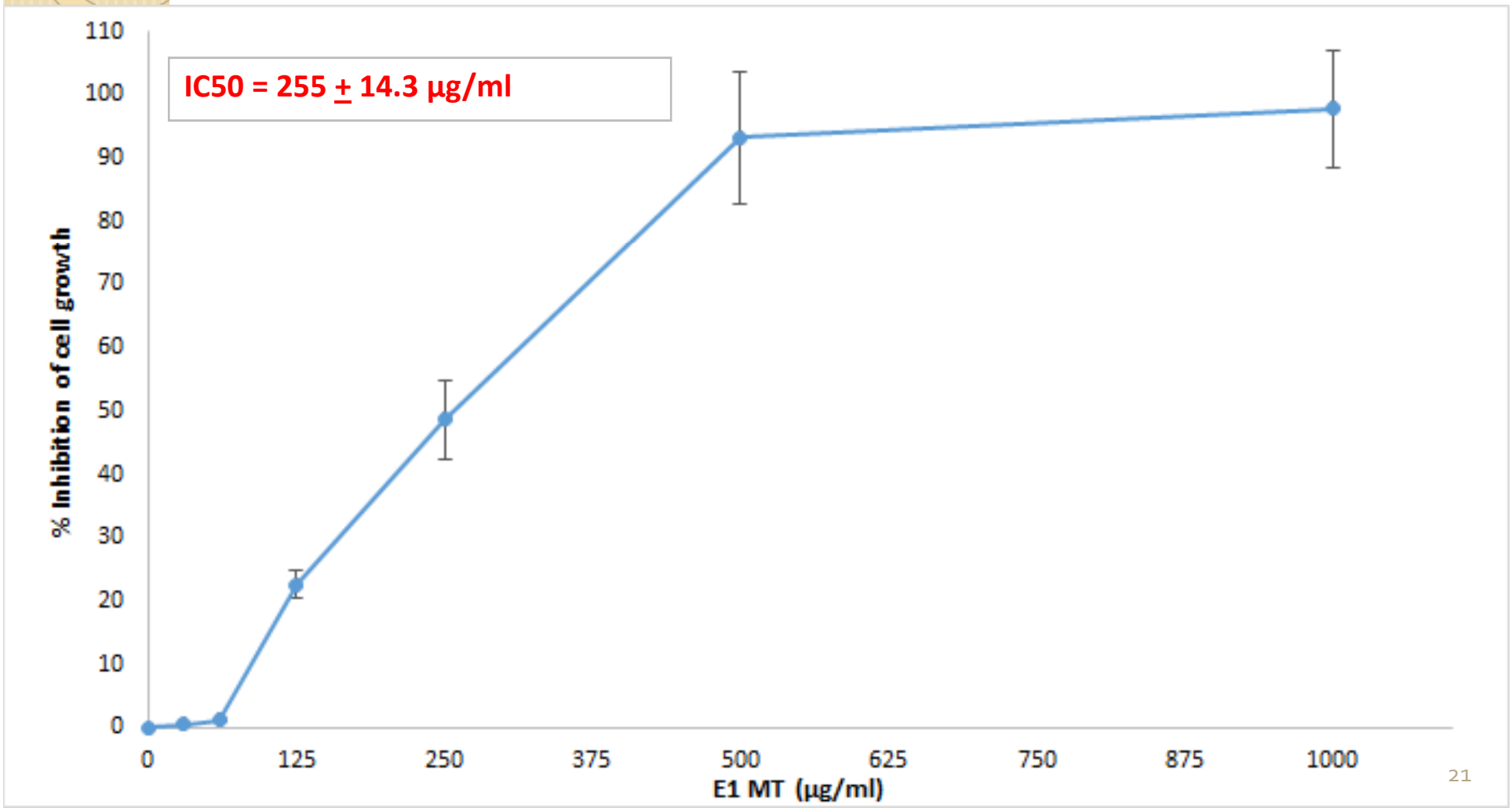
Effect of liposomes NMG extract loaded on the viability of HepG2 cells at various concentrations of liposomes suspensions for 24 h. Results are expressed as percent of viable cells in comparison with the control.

Each experiment was performed in triplicate and the bars represent standard error values.



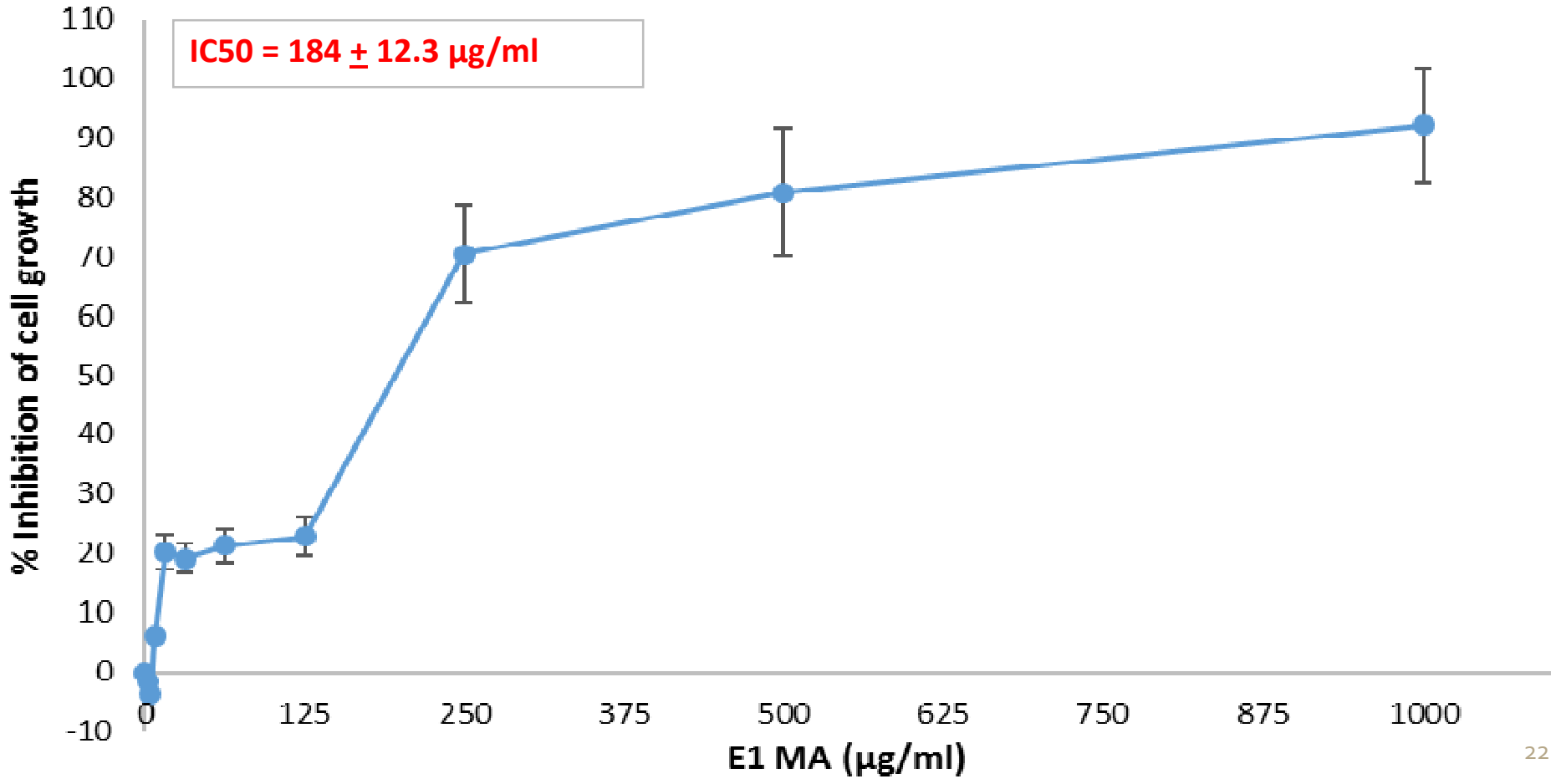
Effect of liposomes TMG extract loaded on the viability of HepG2 cells at various concentrations of liposomes suspensions for 24 h. Results are expressed as percent of viable cells in comparison with the control.

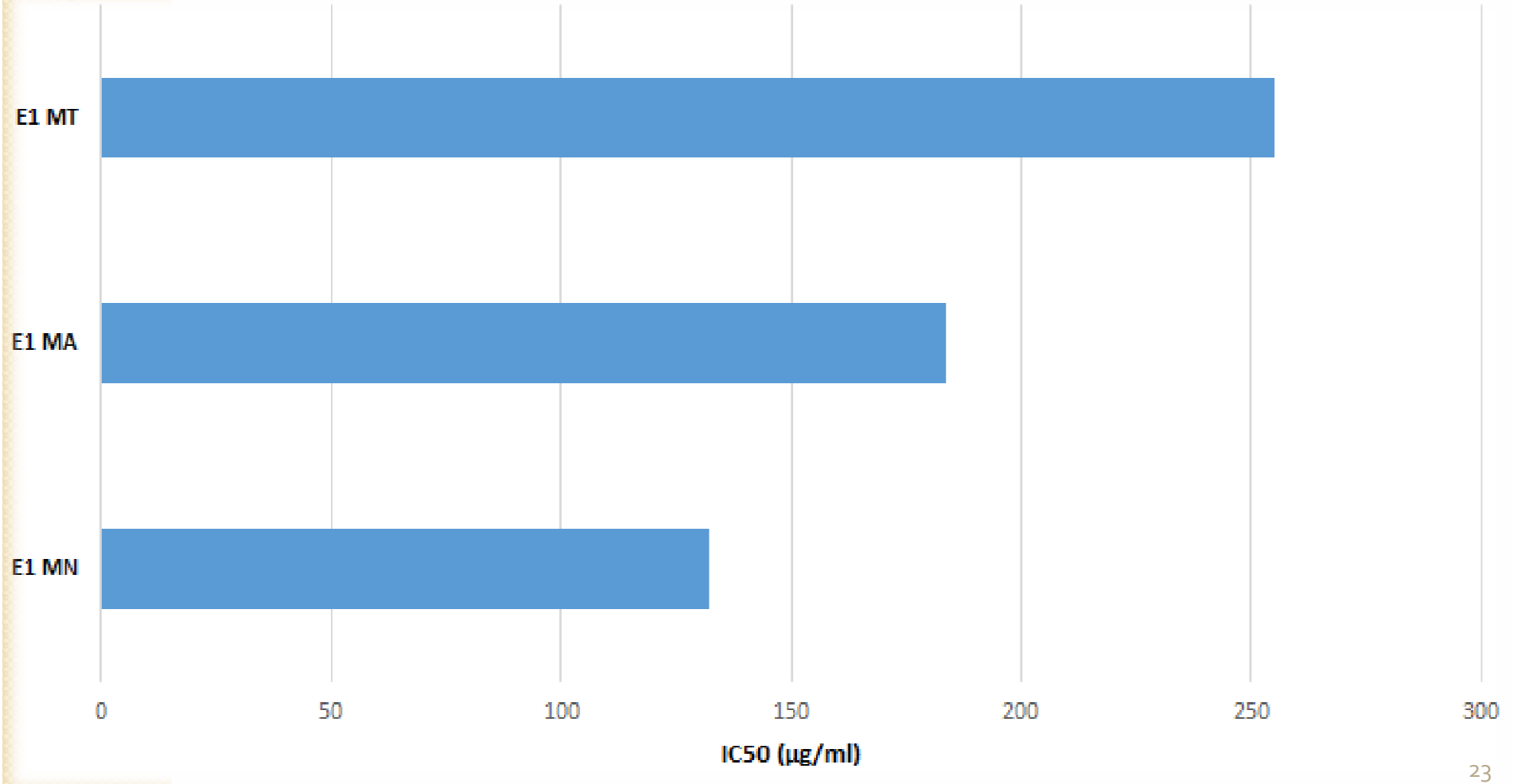
Each experiment was performed in triplicate and the bars represent standard error values.



Effect of liposomes AMG extract loaded on the viability of HepG2 cells at various concentrations of liposomes suspensions for 24 h. Results are expressed as percent of viable cells in comparison with the control.

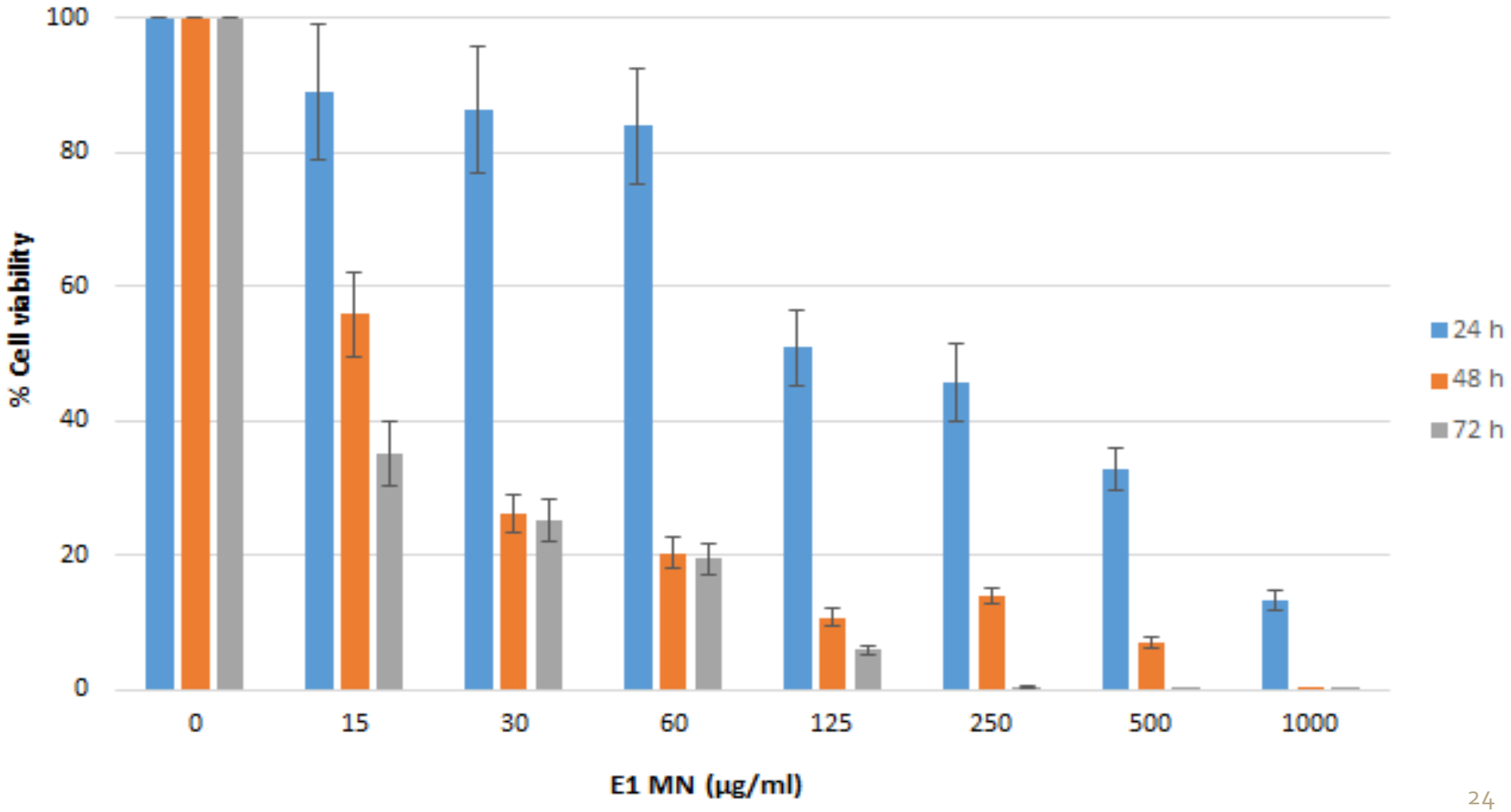
Each experiment was performed in triplicate and the bars represent standard error values.




E1 MT
E1 MA
E1 MN
0
50
100
150
200
250
300
IC50 (µg/ml)

Effect of liposomes NMG extract loaded on the viability of HepG2 cells at various concentration of liposomes suspensions for 24 h, 48h, 72h. Results are expressed as percent of viable cells in comparison with the control.

Each experiment was performed in triplicate and the bars represent standard error values.



In conclusion….

- Colloidal systems containing MGE (N,A,T) were successfully prepared with EI methods. Besides, previous studies proved that EI method was more suitable for entrapment of active agents than other methods.
- The effect of different doses of these recently prepared and characterized liposomes on the growth and viability of HepG2 was evaluated.
- Our results reveal that liposomes MG extract loaded cause a dose and time depended inhibition of survival in malignant HepG2 cells studied.
- When comparing the results of the different liposomal formulations tested, it is clear that liposomes NMG extract loaded were found to be significantly more toxic.
- The inhibitory activity of the extracts against the cell growth indicates that they potentially could be used as chemo protecting agents .
- Nevertheless, further studies are needed to clarify the mechanism by which liposomes MG extracts loaded interact with HepG2 cells (internalization or fusion with the cell membrane).

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Research Article Open Access

Study of Antioxidant and Antimicrobial Activity of Chios Mastic Gum Fractions (Neutral, Acidic) Before and After Encapsulation in Liposomes

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Abstract

Mastic is a well-known natural resin from the trunk and branches, of *Pistacia lentiscus* var. chia (Anacardiaceae), which is grown as endemic only in the Greek island of Chios. During this work, a total mastic gum extract was prepared after removal of the contained insoluble polymer in order to ameliorate solubility and enhance in vivo activity. To overcome the drawbacks (i.e solubility, bioavailability, etc.) of mastic gum extracts (acidic and neutral fraction), the selection of a suitable carrier is crucial. Three different methods of preparation, thin-film evaporation, freezing-thawing, and ethanol injection were used for the preparation of liposomes consisting of Phosphatidylcholine (PC) and Cholesterol (CH). The effect of PC: CH molar ratio on the percentage of mastic extract encapsulated was investigated. Mastic gum extracts components-liposomes interaction was studied using Fourier transform infrared (FT-IR) spectroscopy. The effects of different preparation methods on the physicochemical properties of colloidal systems were evaluated by means of surface morphology, field emission Scanning Electron Microscopy (SEM), and size distribution using a particle size analyzer. For the determination of the antioxidant activity two methods were used: I) The Rancimat method where the protection factor was determined for each sample and compared with known antioxidants. II) Differential Scanning Calorimetry (DSC) where the temperature of oxidation for each sample was determined. Moreover, the crude extract (EtOAc-MeOH) of mastic, as well as, its acidic and neutral fractions was assayed against a panel of 9 human and food pathogenic gram (±) bacteria and fungi.


Thank
you