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D-α-tocopherol-Based Polymeric Micelles for Successful Improving Encapsulation of Retinoic Acid

Guendalina Zuccari[1],* Sara Baldassari[1], Alice Atturo[1], Silvana Alfei[1], Giorgia Ailuno[1], Leonardo Marchitto[2], and Gabriele Caviglioli[1]

[1] Department of Pharmacy (DiFAR), University of Genoa, Viale Cembrano 4, I-16148, Genova, Italy;

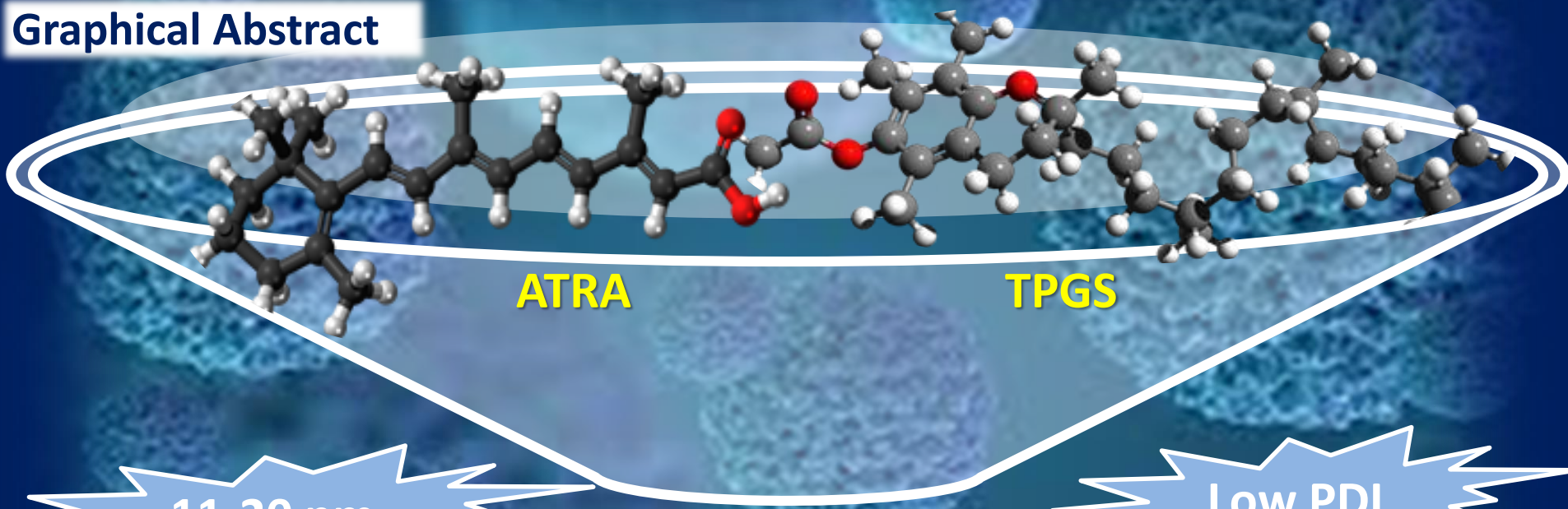
[2] Department of Sciences for the Quality of Life, Corso D'Augusto 237, Rimini, Department of Pharmacy and Biotechnology, Via Belmeloro 6, Bologna, University of Bologna, Italy.

DIPARTIMENTO DI FARMACIA
UNIVERSITÀ DI GENOVA
DIFAR

* Corresponding author: zuccari@difar.unige.it

D-α-tocopherol-Based Polymeric Micelles for Successful Improving Encapsulation of Retinoic Acid

Graphical Abstract

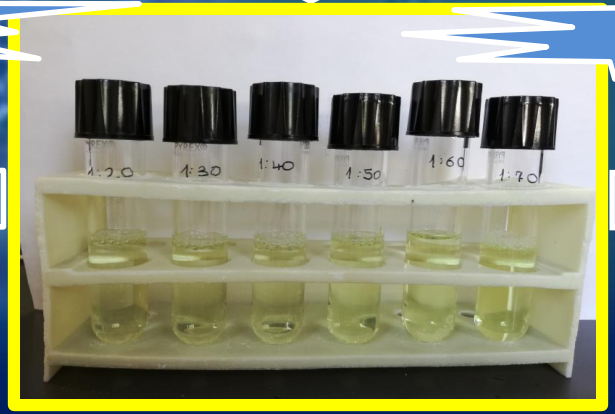


11-20 nm

EE=36-79%

Low PDI

ζ = 0.5-4 mV



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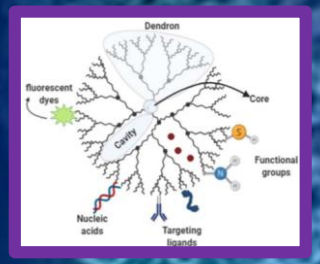
Abstract: From decades, all-trans-retinoic acid (ATRA) has been the first-choice treatment for several skin diseases, including epithelial skin cancer and acne, but its efficacy is strongly limited by its low water solubility and high instability. Different ATRA formulations are commercially available, but their prolonged use leads to loss of effectiveness and causes cutaneous side effects like redness and peeling of the skin. In this work, we studied the capability of D-α-tocopheryl polyethylene glycol succinate (TPGS), a water-soluble derivative of vitamin E able to self-assemble in core-shell nano-aggregates, to encapsulate ATRA, with the aim of overcoming the issues associated with ATRA clinical use. Firstly, this study reports a solubility study based on the equilibrium method, which explored TPGS capability to interact with the host, then ATRA-loaded polymeric micelles (ATRA-TPGSs) were prepared by solvent casting method starting from different TPGS amounts in the preparative mixture. ATRA-TPGSs showed small sizes (11-20 nm), low polydispersity, quite neutral Z potential, and proved good encapsulation efficiency, also confirmed by the FTIR spectra handled by the principal component analysis chemometric tool. The loaded micelles were stable in solution during storage at 25 °C, without the tendency to flocculate or form sediments and proved suitability for freeze-drying. ATRA-TPGSs gel formulations were obtained using Carbopol® as gelling agent and showed a non-Newtonian plastic flow. Further analyses are ongoing to evaluate ATRA-TPGSs skin permeation, and their in vitro cytotoxic effects on melanoma cell lines.

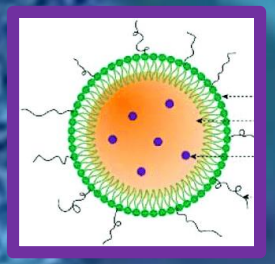
Keywords: ATRA-loaded TPGS-based micelles; enhanced solubility; improved stability; nanosized formulations; low polydispersity.

Introduction

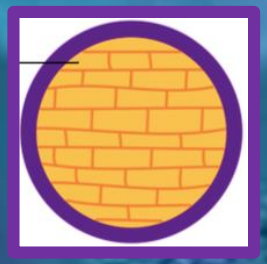
DRUG DELIVERY SYSTEMS



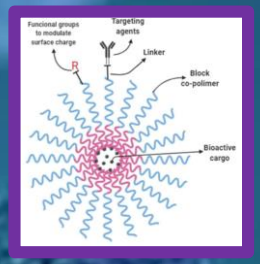
Dendrimer



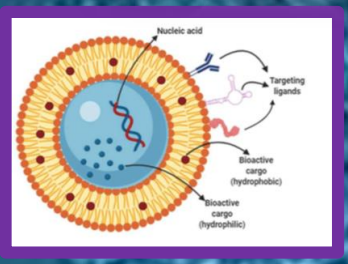
Lipid-Polymer Hybrid Nanoparticle



Solid Lipid Nanoparticle



Polymeric Micelle



Liposome

Nanostructured Lipid Carrier

Modulation of the pharmaco kinetics of an active ingredient

Improvement of the therapeutic efficiency

Improvement of physicochemical stability of the drug to environmental agents

- Light
- High temperature
- Moisture
- Oxygen

Increased shelf life

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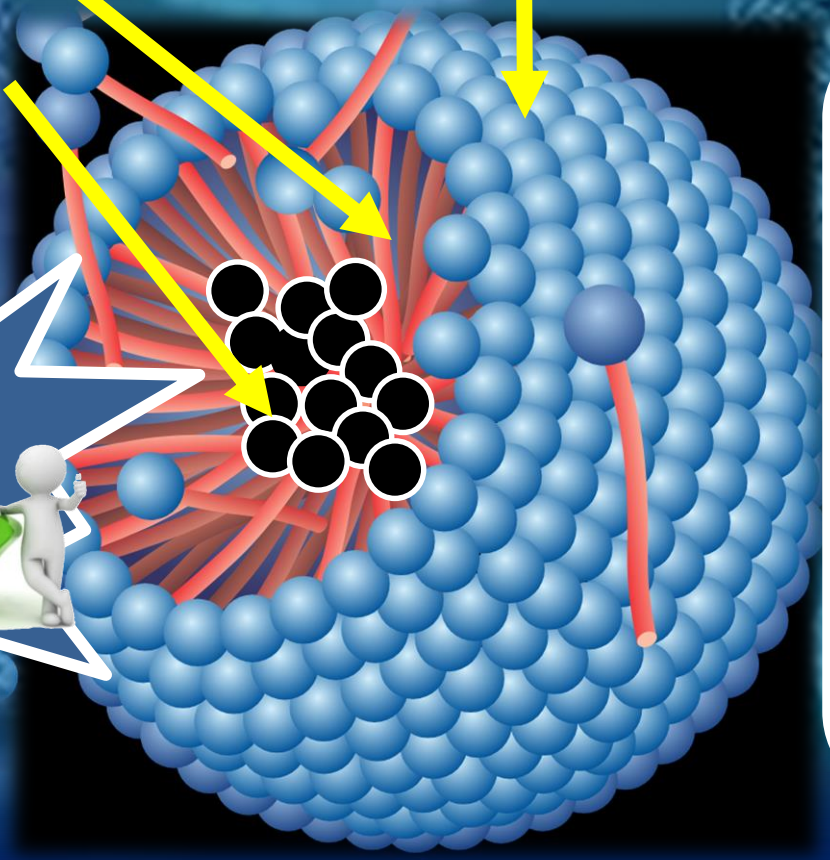
POLYMERIC MICELLES

Hydrophobic *core*
Entrapped
Hydrophobic drug

Hydrophilic *shell*



- Low CMC
- Reduced size (10-200 nm)
- High drug loading (DL%)
- High drug retention
- High riproducibility
- Biocompatibility

Improved
drug
solubility
stability
availability

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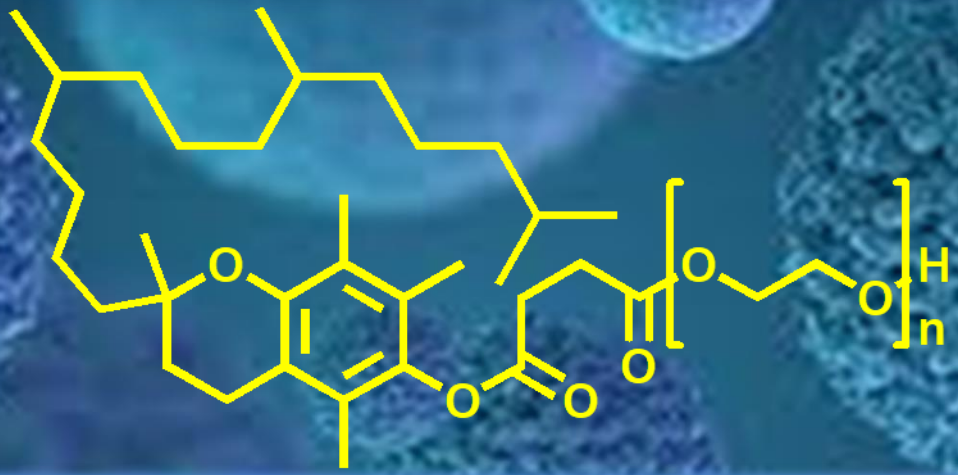
VITAMIN E TPGS



Vitamin E TPGS *(D-α-Tocopheryl polyethylene glycol 1000 succinate)*

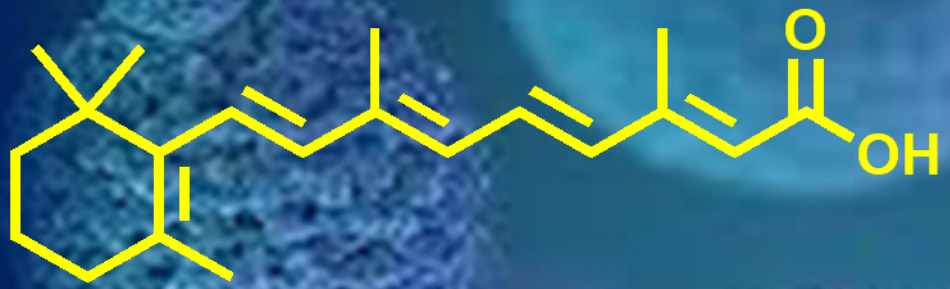
- Syntetic derivative of Vitamin E
- Amphiphilic molecule

- Critical micellar concentration: 0,02 wt%

Uses

- Absorption promoter, drug solubilizer
- Plasticizer or binder
- Vitamin E source

RETINOIC ACID



All-trans-retinoic acid (ATRA)

Marketed Formulations

- Tretinoin Same® cream 0.05%
- Airol®cream 0,05%
- Airol ®cutaneous solution 0,05%

Pharmacological Properties

- Epidermal proliferation and cell turnover
- Comedolysis promotion
- Collagen synthesis
- Melanin Reduction

Topical clinical applications

- *Acne vulgaris*
- Photodamage
- Hyperpigmentation
- Keloid scars and stretch marks
- Psoriasis

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RESTRAIN OF ATRA DRAWBACKS

Adverse reactions

- Erythema
- Burning
- Photosensitization

Poor water solubility (6,3 x 10^{-4} mM)
Chemical instability

- Photodecomposition
- Oxidation
- Thermal Instability

- Reduction of Cutaneous Irritation
- Improvement of patient *compliance*
- Enhancement of absorption and skin retention

DRUG DELIVERY SYSTEMS

- Improvment of solubility
- Protection from chemical degradation
- Enhancement of shelf life

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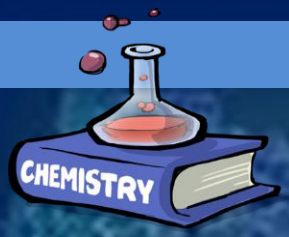
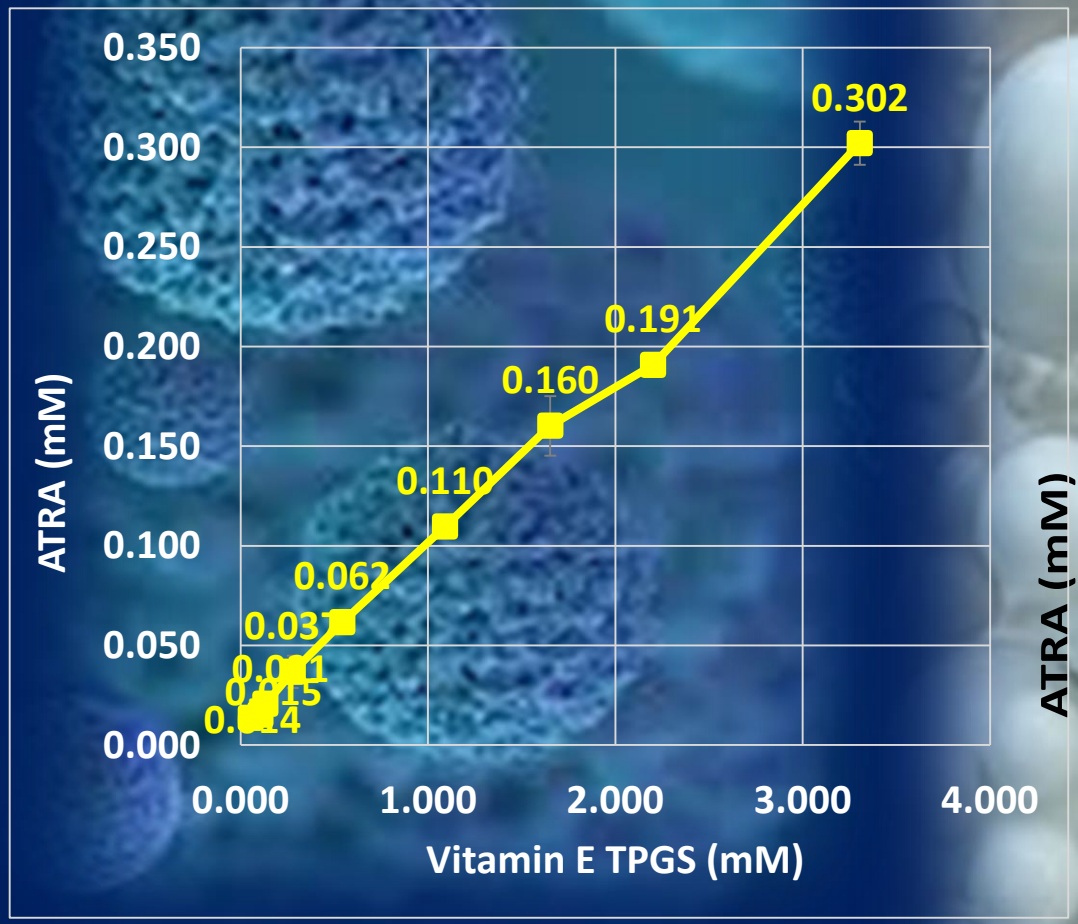
PHASE SOLUBILITY STUDIES





Shake-flask Method

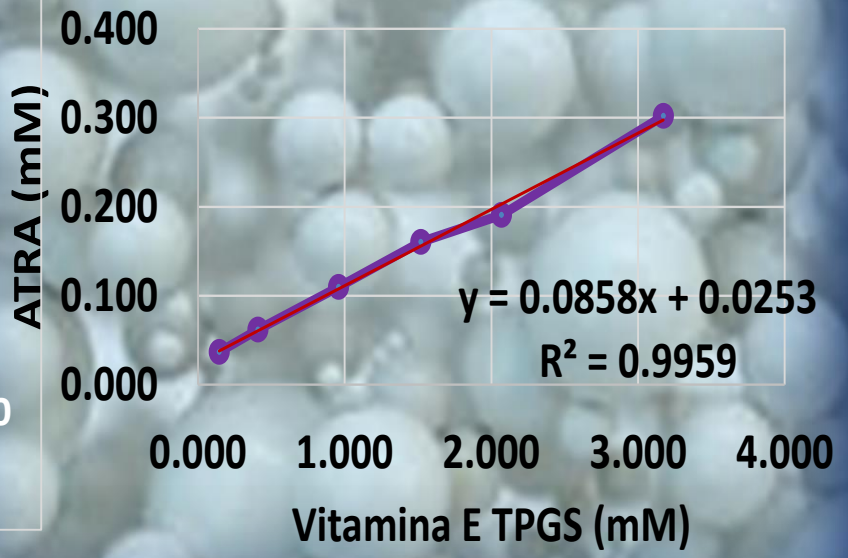
ATRA in micelle

↓↑

ATRA in solution

↓↑

ATRA Residual



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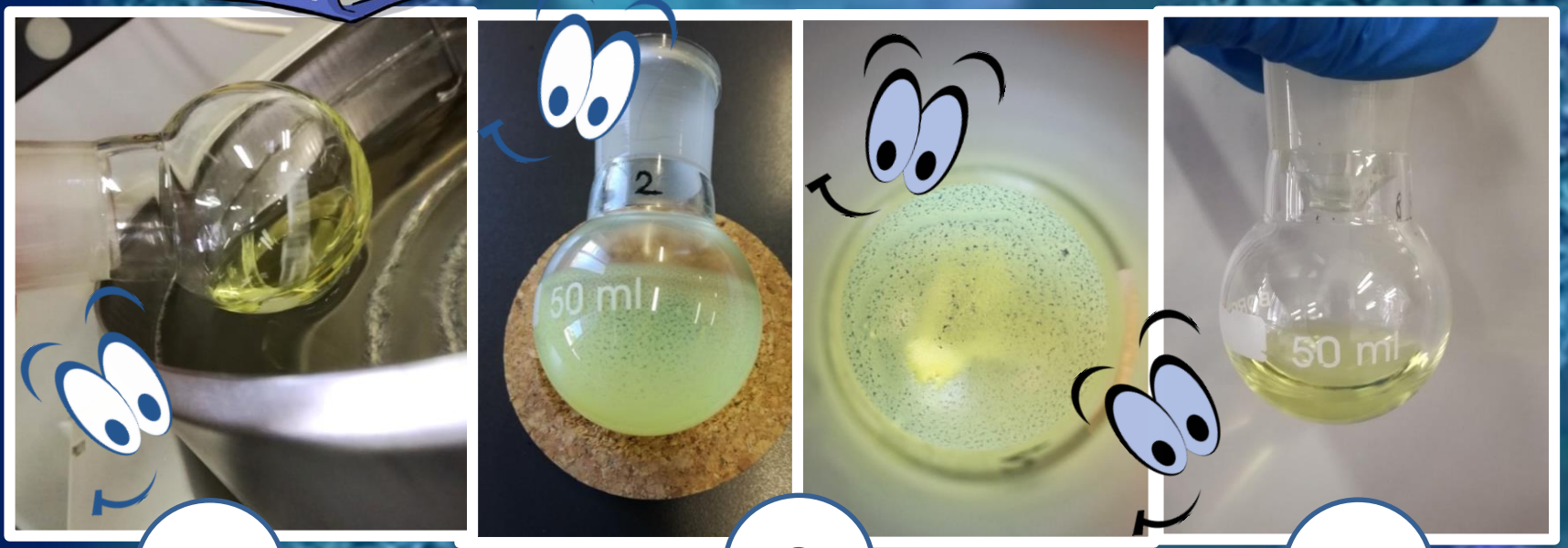
PREPARATION OF THE MICELLES

Solvent Casting Method



1 ATRA and Vitamin E TPGS Solution in Ethanol

Solvent removal

2 Formation of the thin layer

Hydratation

3 Micellar Suspension

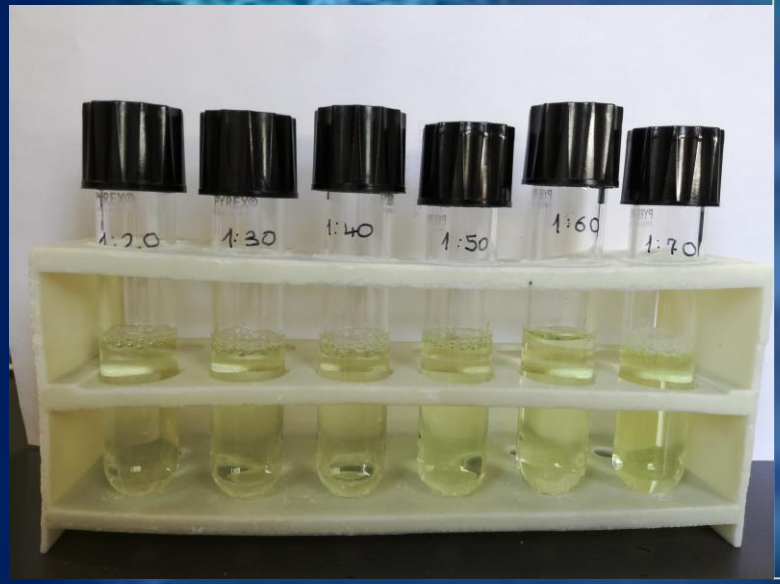
1)Filtration

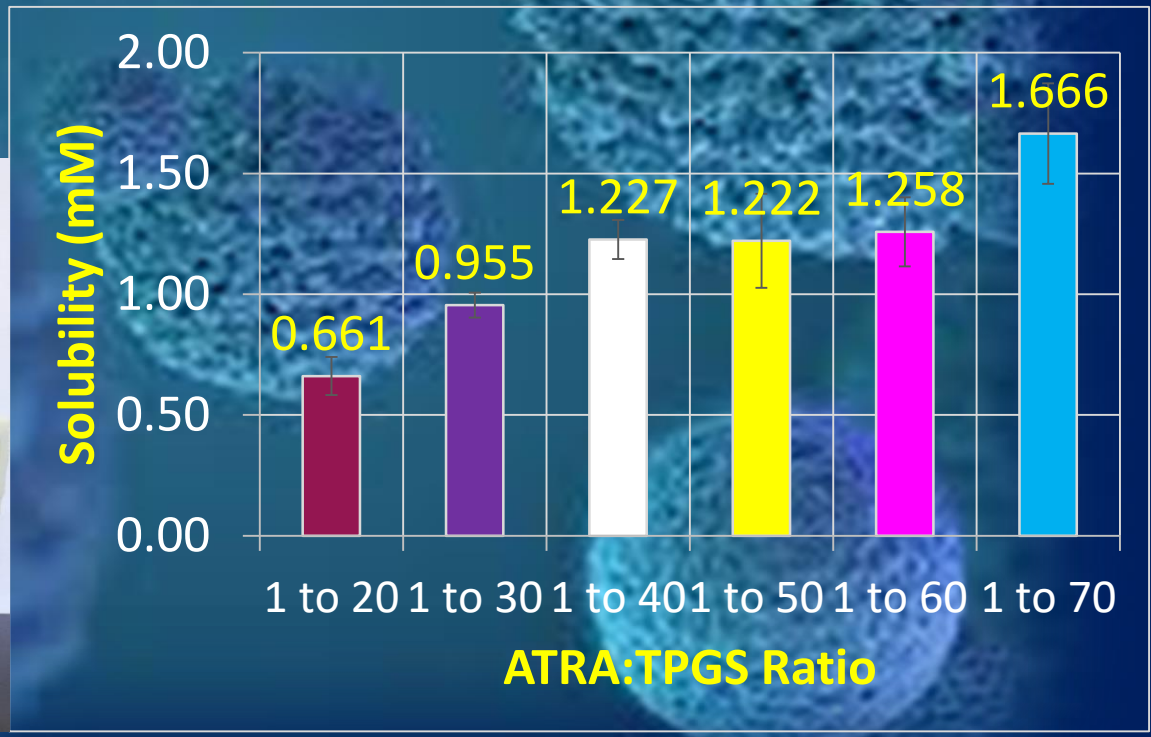
2)Analysis

Results and discussion

ATRA:TPGS RATIO OPTIMIZATION

ATRA:TPGS in the preparative Mixture
1:20, 1:30, 1:40, 1:50, 1:60, 1:70





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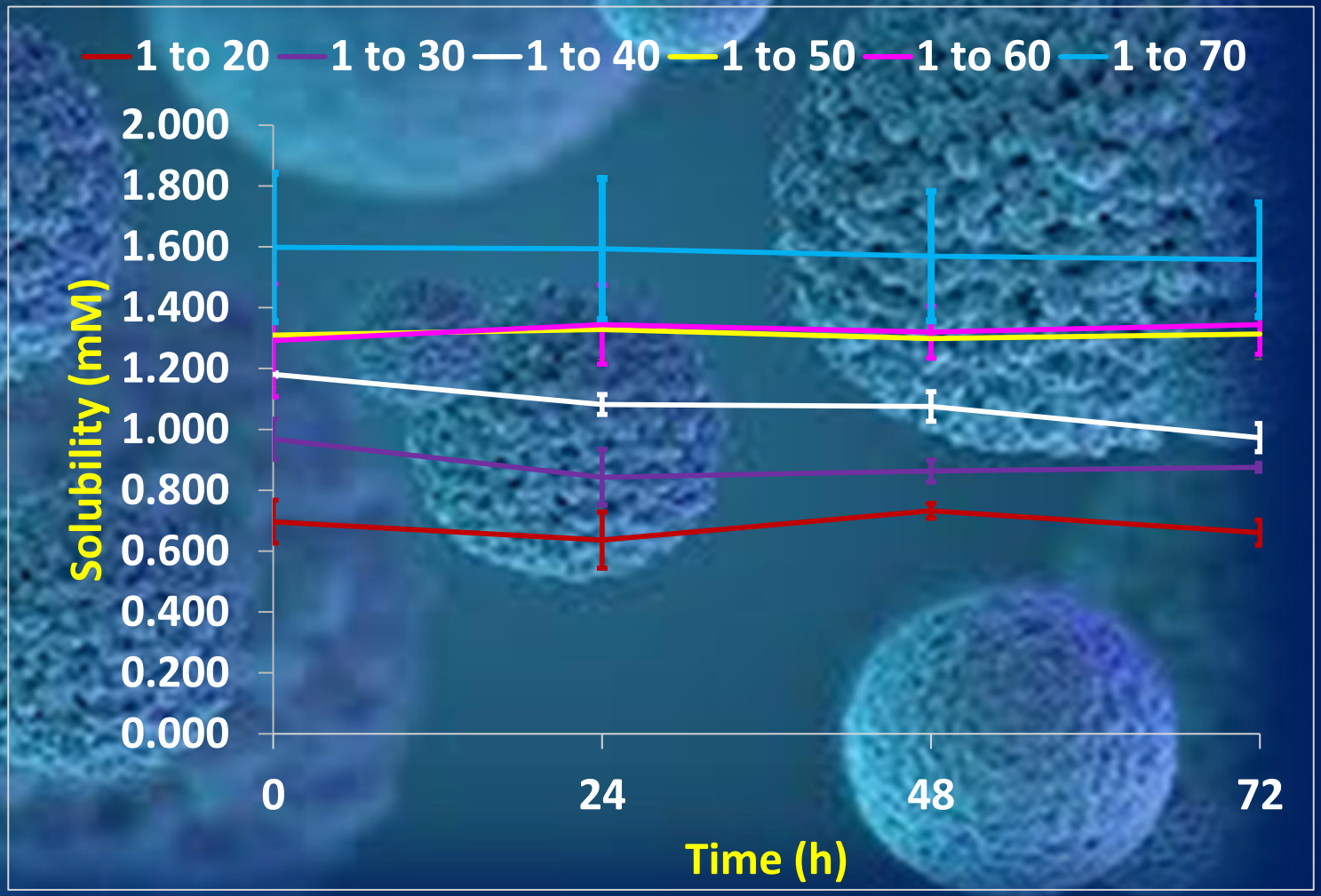
STABILITY STUDIES

- Micellar solutions in Incubator at 25°C
- Analysis at 0, 24, 48 e 72 h
- Filtration with 0,45 µm membrane
- Absorbance measueres at 345 nm



Best Preparative Mixture 1:50

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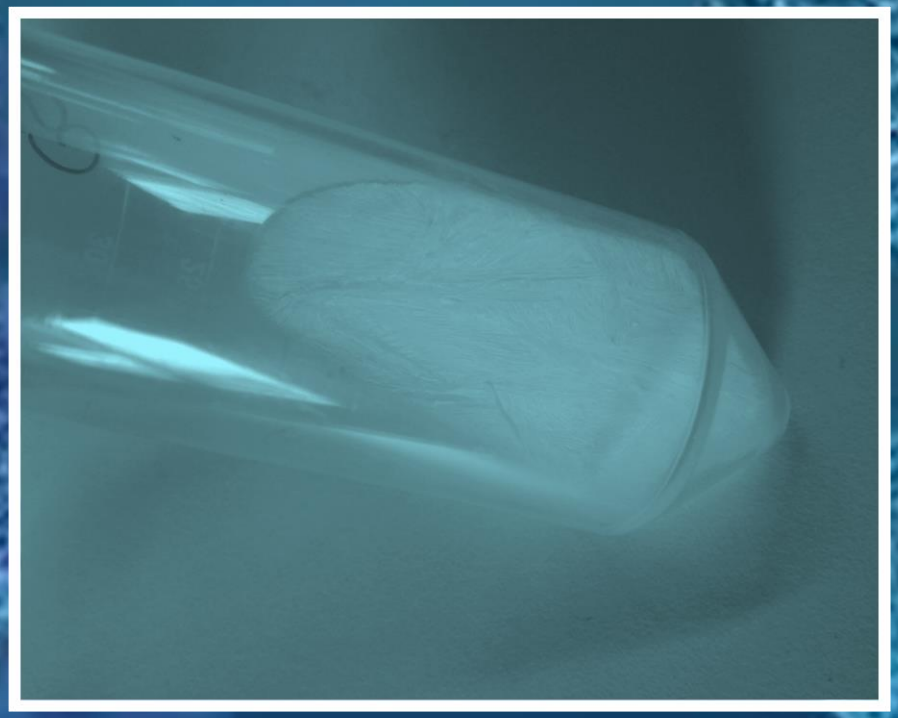
FREEZE-DRYING

1. Flute beak pre-freezing in a freezer at -20 ° C
2. Cooling at -30 ° C in the freeze-drying chamber
3. Primary drying at 20 x 10^{-3} mBar for 48 h
4. Secondary drying at 25°C for 1 h

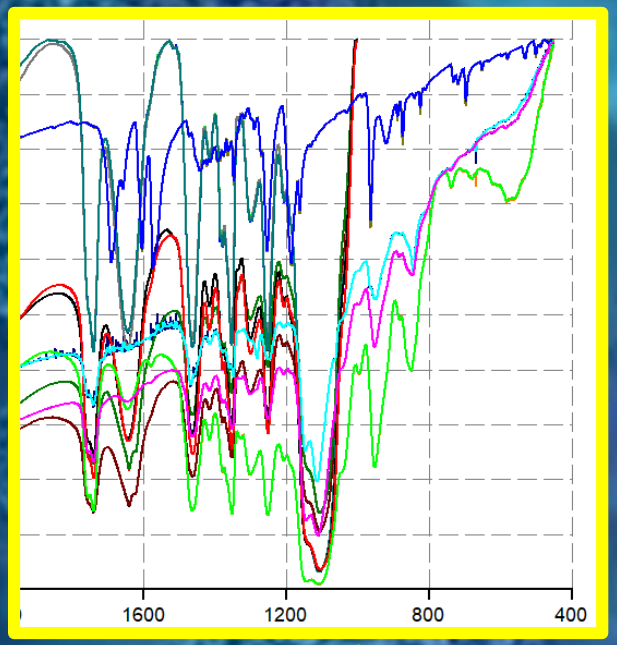


ADVANTAGES

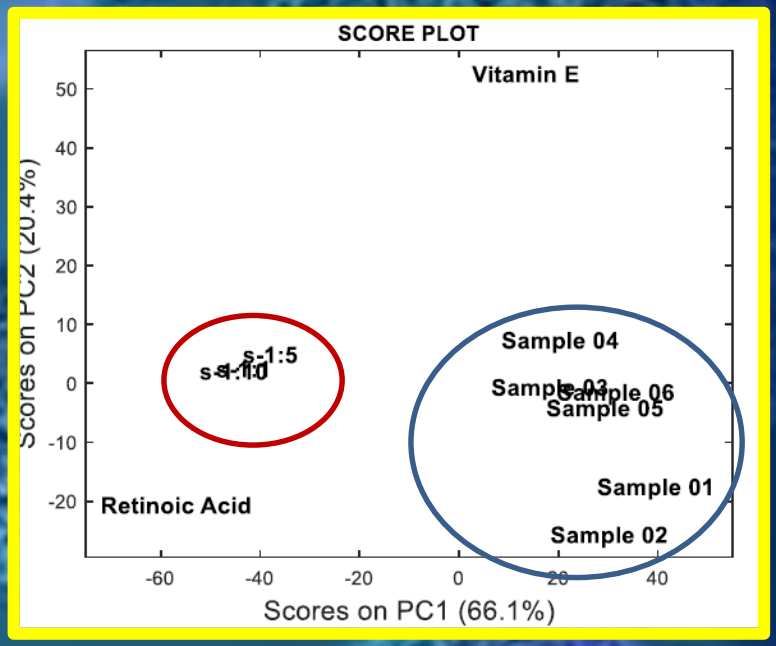
- ✓ Drug stabilization inside the micelle
- ✓ Extended shelf life
- ✓ Dimensions of the micelles unchanged after reconstitution of the powder

Pale yellow spongy solid easily resuspendable

FTIR PCA-ASSISTED ASSESSMENT OF THE SUCCESSFUL ENCAPSULATION



Significant bands of FTIR spectra of samples of the micelles of this study and of three additional ones having a high content of ATRA prepared as reference (1:1, 1:5, 1:10)

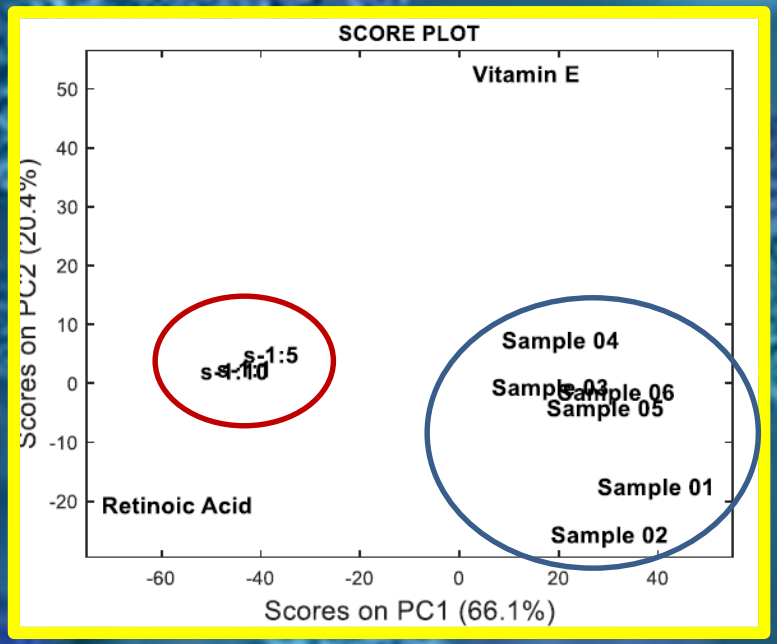


Score Plot of PCA on the FTIR data of all samples, ATRA and Vitamin E TPGS

FTIR spectra were considered in absorbance scale

Data were processed after Standard Normal Variate Trasform (SVN) and column centering

PCA (absorbance scale, SVN, column centering)



Micelles samples, ATRA and Vitamin E TPGS appeared well separed on PC2

The location of all samples close to ATRA rather than to Vitamin E TPGS confirmed the presence of ATRA inside the complexes

The total samples population appeared furtherly separated on PC1

The location of samples circled in red closeer to ATRA than those circled in blue confirmed their higher content in ATRA (1:1, 1:5, 1:10) in respect of samples 01-06 of the present work (1:20, 1:30, 1:40, 1:50, 1:60, 1:70)

ENCAPSULATION EFFICIENCY AND DRUG LOADING

$$EE\% = \frac{Wt}{Wi} * 100$$

It expresses the quantity of drug encapsulated by the nanocarrier in respect to that used in the preparative mixture

ATRA:TPGS	EE%
1 to 20	35,5 ± 2,2
1 to 30	47,4 ± 3,3
1 to 40	58,6 ± 0,2
1 to 50	64,8 ± 8,0
1 to 60	64,3 ± 9,4
1 to 70	79,1 ± 11,3

$$DL\% = \frac{Wt}{Wn} * 100$$

It expresses the quantity of drug encapsulated by the nanocarrier in respect to the mass of the ATRA-TPGS complex

ATRA:TPGS	DL%
1 to 20	3,80 ± 0,04
1 to 30	3,74 ± 0,87
1 to 40	3,71 ± 1,12
1 to 50	3,75 ± 0,67
1 to 60	3,47 ± 0,73
1 to 70	4,22 ± 0,30

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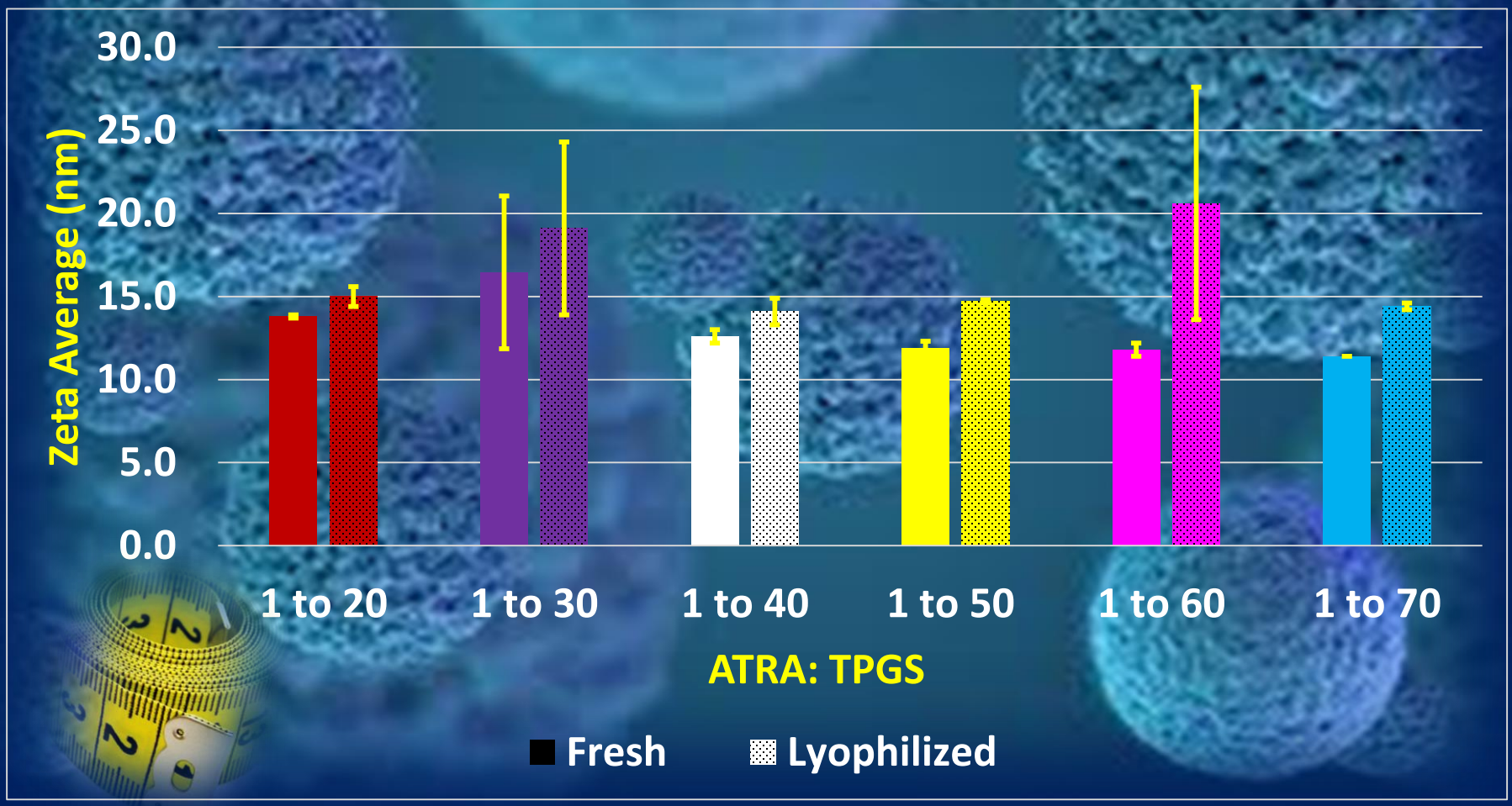
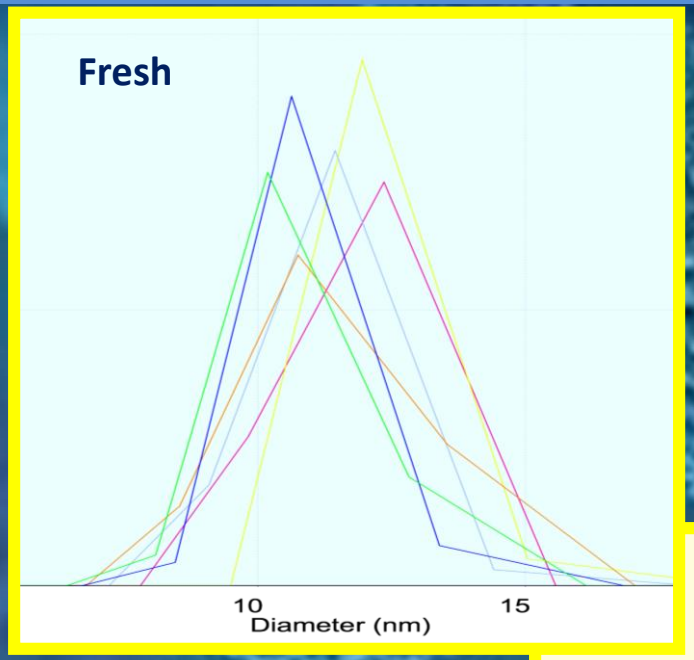
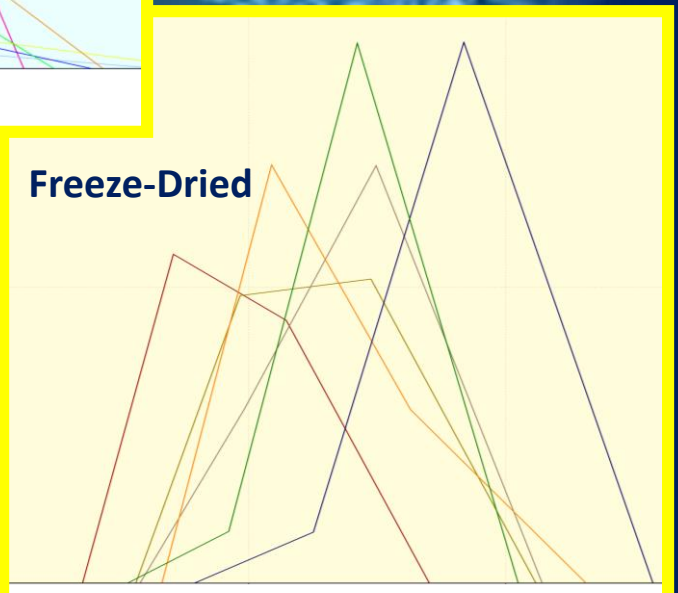
DIMESIONAL ANALYSIS BY DYNAMIC LIGHT SCATTERING (1)



DIMESIONAL ANALYSIS BY DYNAMIC LIGHT SCATTERING (2)

ATRA:TPGS	PDI Fresh Micelles	PDI Freeze-Dried Micelles
1 to 20	0,238	0,324
1 to 30	0,273	0,314
1 to 40	0,167	0,213
1 to 50	0,126	0,212
1 to 60	0,137	0,364
1 to 70	0,114	0,193





ZETA POTENTIAL (ζ)

ATRA:TPGS	1 to 20	1 to 30	1 to 40	1 to 50	1 to 60	1 to 70
ζ	-7,2	-9,3	-12,6	-10,8	-13,2	-10,1

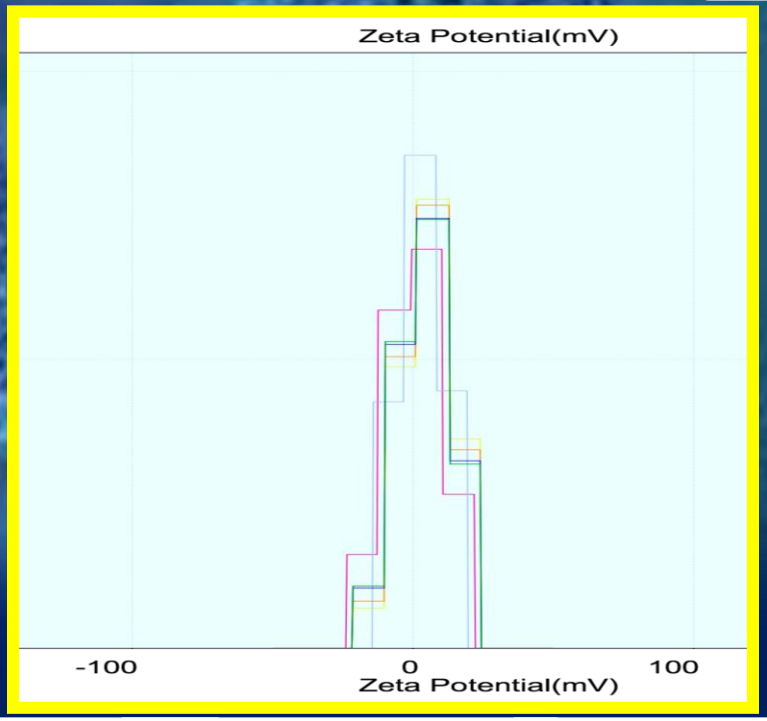
ζ (H_2O mQ)
Mean Value = -10,5 mV

→ Shelf life

ATRA:TPGS	1 to 20	1 to 30	1 to 40	1 to 50	1 to 60	1 to 70
ζ	-0,5	5,1	-0,4	5,0	4,1	4,0

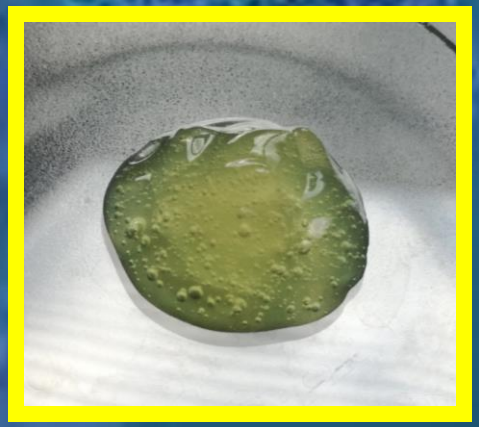
ζ (HEPES Buffer 5mM)
Mean Value = 2,9 mV

→ Stability in vivo



GEL PREPARATION

1. Addition of increasing amounts of Carbopol ® 980 NF to the colloidal solution ATRA:TPGS 1:50
   - 0,5%
   - 1,0%
   - 1,5%
2. Carbopol hydratation for 120 min
3. Neutralization with tryethanolamine



Carbopol ® (%)	pH
0,5 %	5,8
1,0 %	5,1
1,5 %	5,0

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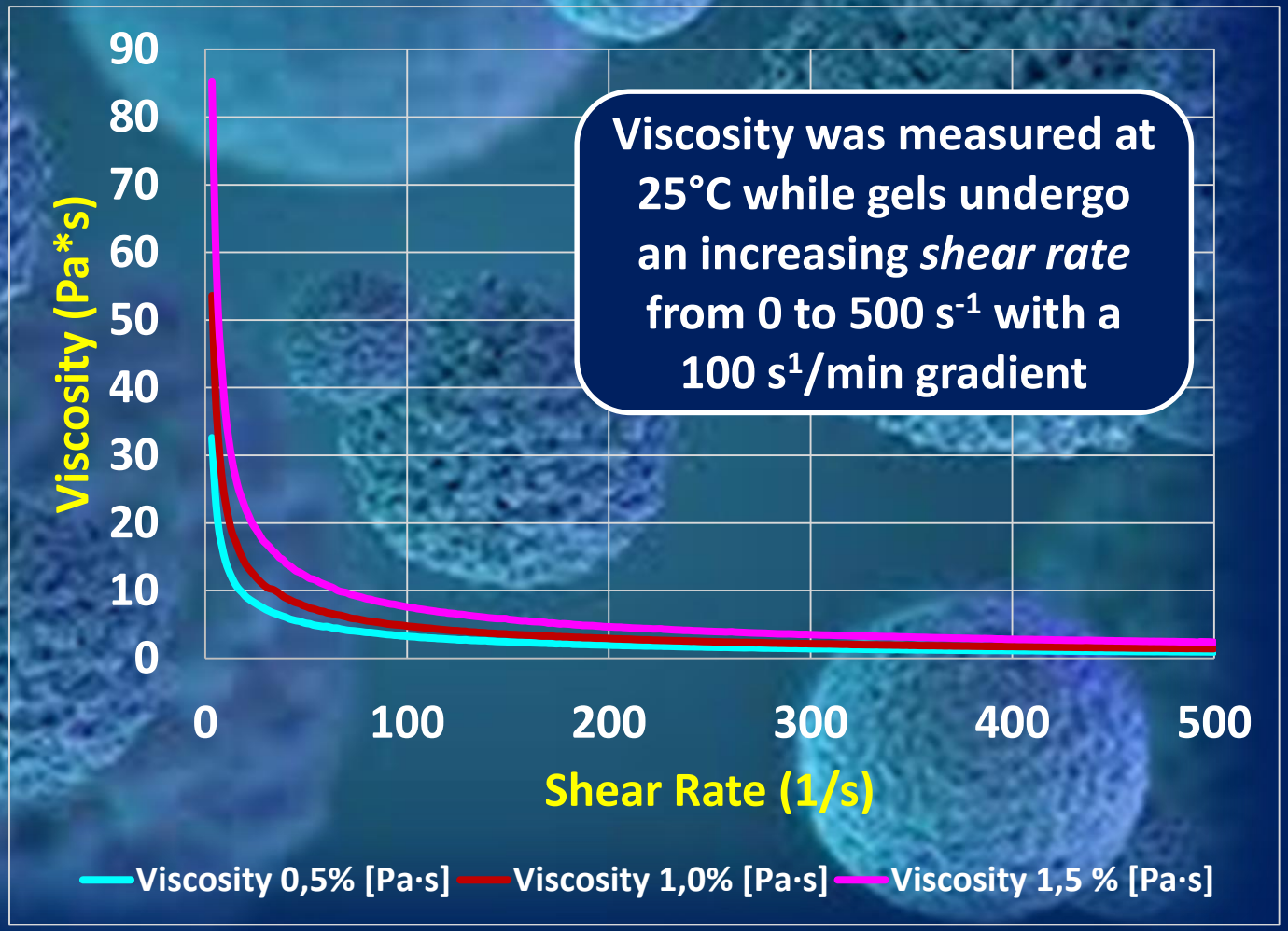
STUDY OF GELS RHEOLOGICAL PROPERTIES (1)

Viscosity varied as a function of the Shear Rate

Fluids with a SHEAR THINNING *behaviour*

Viscosity increased with the improvment of the concentration of the gelling agent

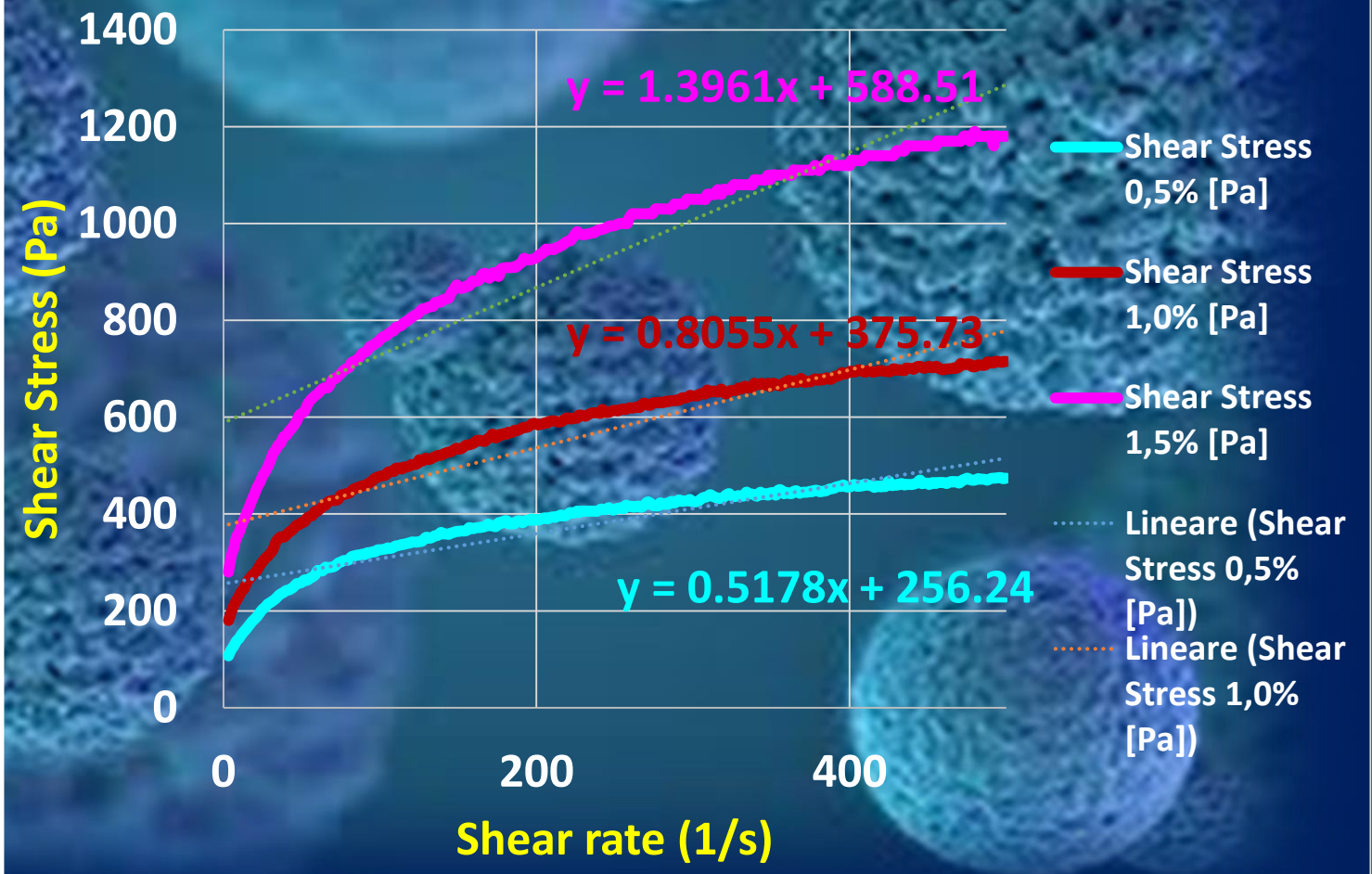


STUDY OF THE GELS RHEOLOGICAL PROPERTIES (2)

The slip started after a certain shear stress value (yield value)

PLASTIC FLUIDS

Different Yield values on the base of the concentration of the gelling agent (Bingham Model)



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CONCLUSIONS

SOLUBILITY

ATRA solubility was increased from 6,3 x 10^{-4} mM to 1,22 mM

SIZE

Micelles with low size (16-20 nm) and low polydispersity (PDI < 0,3) were achieved

STABILITY

Good stability was obtained
No aggregates or residuals formation was observed after 72 h
Materials suitable for freeze drying processes were prepared

GELS

Not Newtonian gels with plastic behaviour and isoepidermic pH were obtained

FUTURE PERSPECTIVE

DEGRADATION

Studies concerning ATRA stability and determination of its possible degradation products by HPLC analysis

PERMEATION

Studies concerning ATRA release and skin permeation using an in vitro model (Franz cell)

BIOACTIVITY

Studies to establish the cytotoxic effects of ATRA-loaded micelles on melanoma cells lines

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