Effects of Ionizable and Non-ionizable Excipients on Lyophilized RNA Formulations Using FTIR-ATR Technology

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USP - Collaborating to pave the way for mRNA-based vaccines and therapeutics quality

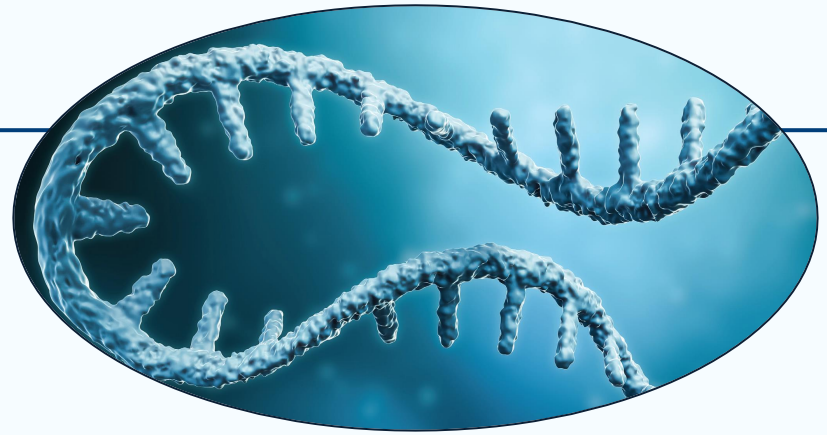
Agenda

PerkinElmer
For the Better

- Why RNA?
- mRNA Formulations
- Not only API - Excipients
- How to characterize excipients compatibility – FTIR Spectroscopy
- Effect of NaCl and Sucrose on mRNA lyophilized formulation
- Effect of Cationic Lipids on mRNA lyophilized formulation
- Conclusion

Why RNA?

- RNA-based therapeutics **fix the pathology** instead of treating the symptoms.
- Specific **regulate disease-causing genes** and their variants.



- **Personalized** medicine.
- Once established delivery and nucleic acid chemistry, relatively **fast** and **effective** production for new target.

RNA-based approved therapeutics

1998

Fomivirsen

2013 – 2016

Mipomersen
Eteplirsen
Nusinersen

2018 – 2023

Inotersen
Golodirsen
Viltolarsen
Casimersen
Patisiran
Givosiran

Lumasiran
Inclisiran
Vutrisiran
Tozinameran
Elasomeran

At present, many mRNA vaccines are in **clinical trials** for different diseases, including **influenza**, **cardiovascular** diseases, **cancer**, **rabies** and **cystic fibrosis** and more.

- 8 ASO (antisense oligonucleotides)
- 5 siRNA (small interfering)
- 2 mRNA vaccines

Wang, X., & Jin, H. (2022). Cell Death & Disease, 13(7), 644.
Bajan, S., & Hutvagner, G. (2020). Cells, 9(1), 137.
Jo, S. J., Chae, S. U., Lee, C. B., & Bae, S. K. (2023). International Journal of Molecular Sciences, 24(1), 746.

mRNA Formulations

Naked mRNA has been mainly applied ***ex vivo*** by using physical methods, including electroporation, microinjection and gene gun.

In ***in vivo*** applications, intravenously administered **naked mRNA** is rapidly **degraded** by **ribonucleases** and the innate **immune system** can be **activated**.

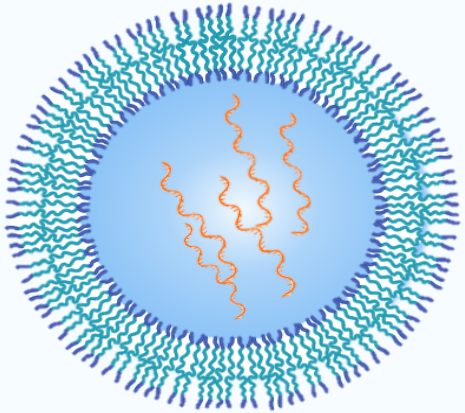
The half-life of naked mRNA has been estimated **<5 min** after **intravenous administration.**

Need for Formulation

- **Avoid degradation** from extracellular degradative agents such as ribonucleases
- **Interact** with the **target cell**
- **Cross the cytoplasmic membrane** and diffuse in the cytoplasm to reach the ribosomes

mRNA Formulations

- **Lipid-based** systems
- **Polymeric** Systems
- **Polypeptidic** systems
- **Other Systems** (Dendrimers, Gold NPs, Combined systems …)



Not Only API – Excipients

PerkinElmer

Nucleoside-modified mRNA encoding the viral spike (S) glycoprotein of SARS-CoV-2

Storage −80 °C to −60 °C

4284 nucleotides

Lipids

- 2[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
- (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
- 1,2-distearoyl-sn-glycero-3-phosphocholine
- Cholesterol

Salts

- Sodium chloride
- Potassium chloride
- Monobasic potassium phosphate
- Dibasic sodium phosphate dihydrate

Other

- Sucrose

Pfizer BIONTECH

Source FDA

Storage −20 °C

4004 Nucleotides

Lipids

- PEG2000-DMG: 1,2-dimyristoyl-rac-glycerol, methoxypolyethylene glycol
- 1,2-distearoyl-sn-glycero-3-phosphocholine
- Cholesterol
- SM-102: heptadecane-9-yl8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy) hexyl) amino) octanoate

Salts

- Tromethamine
- Tromethamine hydrochloride
- Acetic acid
- Sodium acetate

Other

- Sucrose

moderna

Not Only API – Excipients

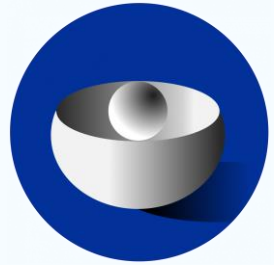
PerkinElmer

FDA

"Any **active ingredients** that are **added intentionally** to therapeutic or diagnostic products, but they are not intended to exert therapeutic effects at the intended dosage, although they **may act to improve product delivery**"

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

"An excipient is a **constituent** of a medicine **other than the active substance**, added in the formulation for a **specific purpose**"



What?

Inorganic or **organic** in composition, **synthetic** or **semi-synthetic**, or derived from **biological** or **natural sources**.

Why?

Overcome limitations of the API, such as low solubility, permeability and stability. (e.g., **enhance absorption, control release** and **improve stability** of the drug substance).

How?

Excipients may constitute **over 50%** of **solid** dosage forms and over **90%** of **liquid** dosage forms.

mRNA / Nanomedicine Debate on Excipients

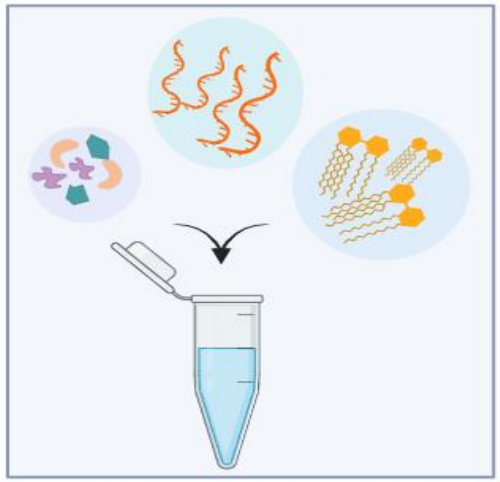
Spikevax Case

FDA accepted classification of PEG2000-DMG and SM-102 as 'starting materials' for the drug substance, rather than as excipients.

EMA regarded all four lipid components of the LNP as excipients.

Hemmrich, E., & McNeil, S. (2023). Active ingredient vs excipient debate for nanomedicines. *Nature Nanotechnology*, 1-4.

Excipients Compatibility Studies



- ***Chemical Interaction***
- ***Physical Interaction***
- ***Physiological Interaction***

- **Thermal Analysis**: DSC/TGA/Hot Stage Microscopy, Isothermal Microcalorimetry
- **Spectroscopy:** FTIR, Raman, Solid State NMR, Powder X-Ray Diffraction
- **Chromatography**: SIC, TLC, HPLC, LCMSMS

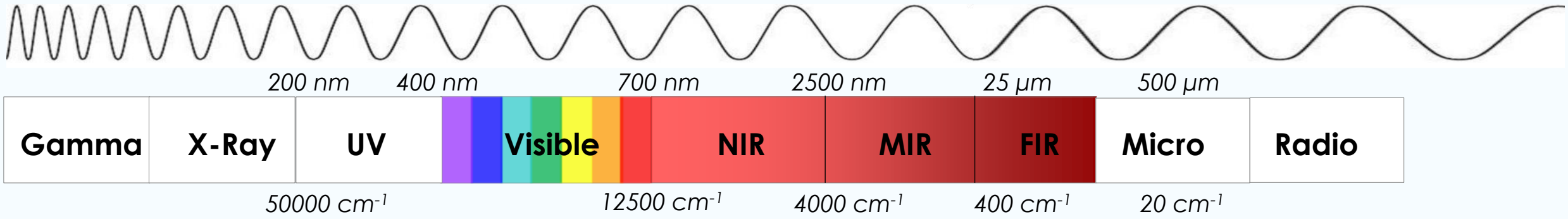
Often combined with IST (Isothermal Stress Testing)

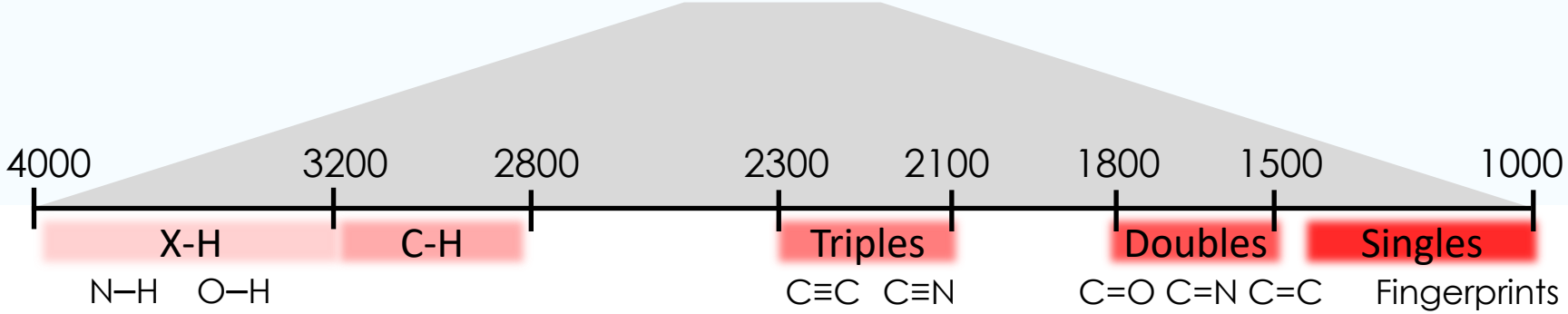
FTIR Spectroscopy

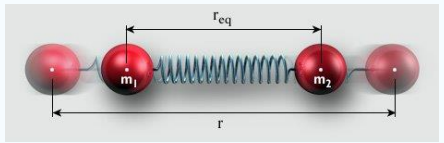
- Easy samples preparation
- Rapid Analysis
- Common Quality Control equipment
- Useful for API without melting point
- Detects changes in the crystal structure, i.e. polymorphic changes, desalting, and degree of hydration
- Detects formation/break of chemical bonds, environmental changes, conformational changes

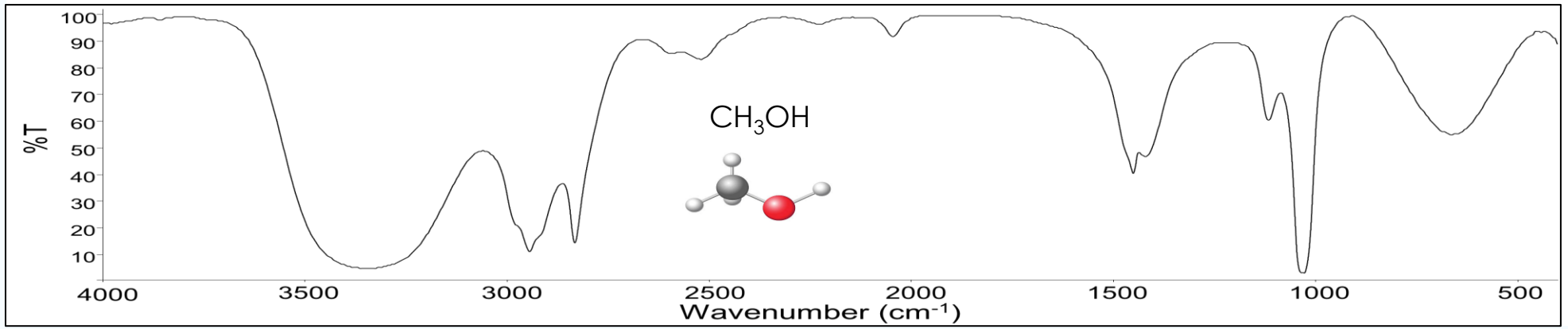
- Spectral Interference – it might require the application of statistical analysis for data interpretation
- Water sensitivity

FTIR – Vibrational Spectroscopy

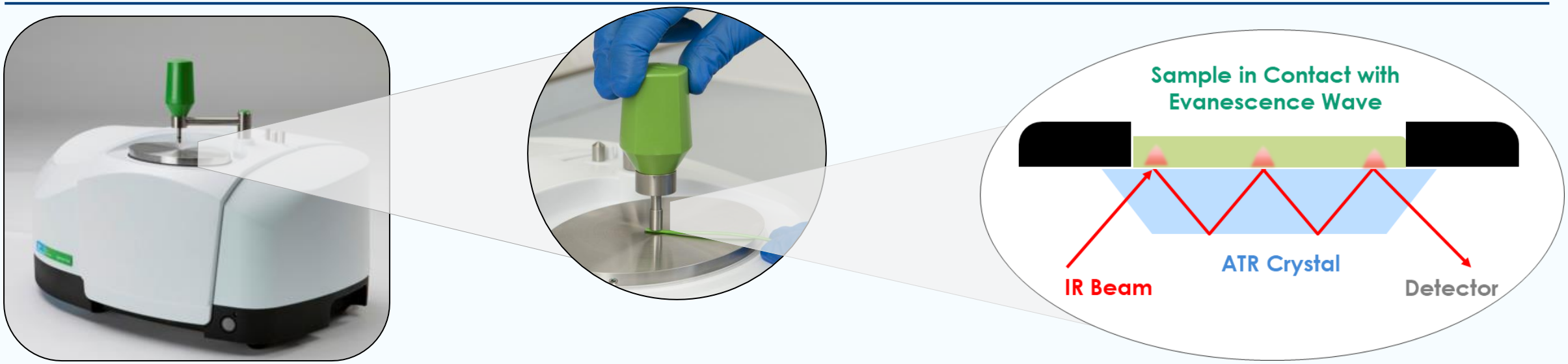








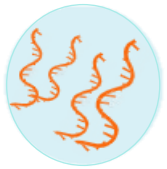
FTIR-ATR Attenuated Total Reflectance



- **Universal** – A variety of samples can be measured – ideal for thick or strongly absorbing samples
- **Direct** – Analysis of samples in their natural states – no need to heat, press into pellets, or grind
- **Non-Destructive** – Solid and liquid samples without altering their properties
- **High Sensitivity** – Trace amounts can be detected

- **Surface analysis –** Limited to a penetration of few microns
- **Spectral artifacts –** Crystal temperature, pressure, and contact force can affect spectra accuracy

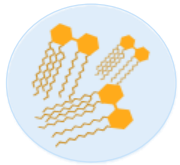
RNA – Excipients Preparation



RNA

From the yeast Saccharomyces cerevisiae (average of 100 nt)

+



Cationic Lipid

DOTAP (1,2-dioleoyloxy-3-(trimethylammonium)propane)Chloride

+

NaCl

Sucrose

RNA : Sucrose

1:2 | 1:1 | 2:1

RNA : NaCl

1:2 | 1:1 | 2:1

DOTAP : RNA : Sucrose

1:2:2 | 1:1:1

DOTAP : RNA : NaCl

1:2:2 | 1:1:1

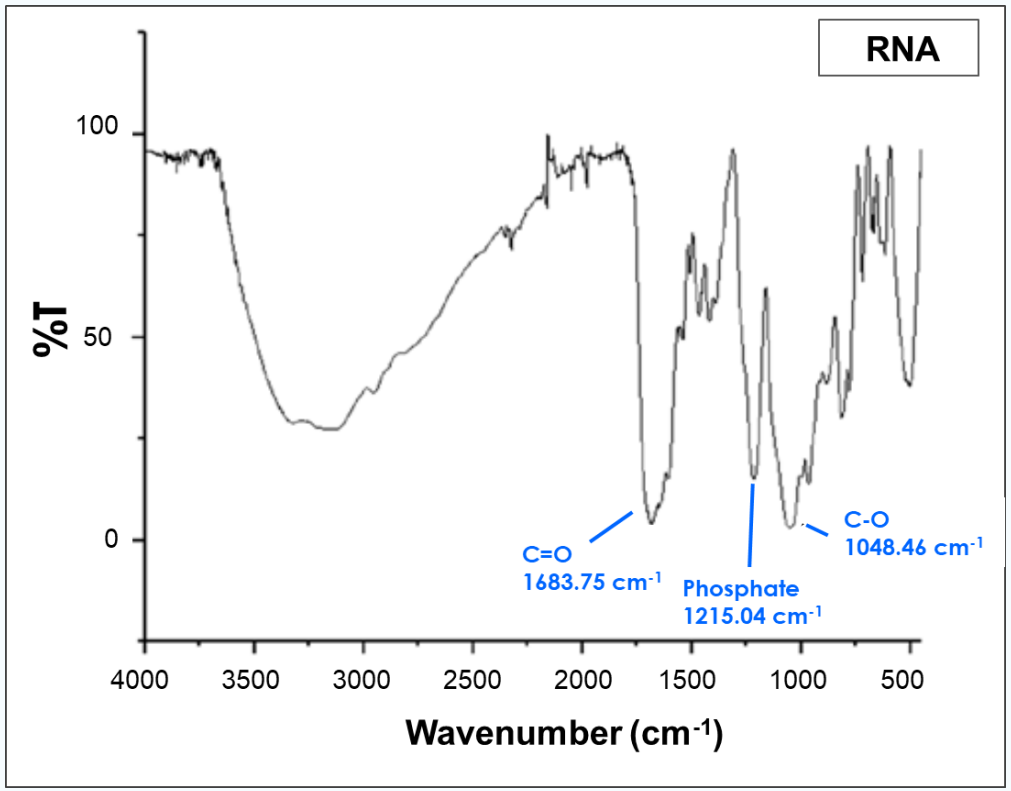
Lyophilization

- Freezing -40°C 6h
- Primary Drying -35°C 26h 0.130 mbar
- Secondary Drying 25°C 8h

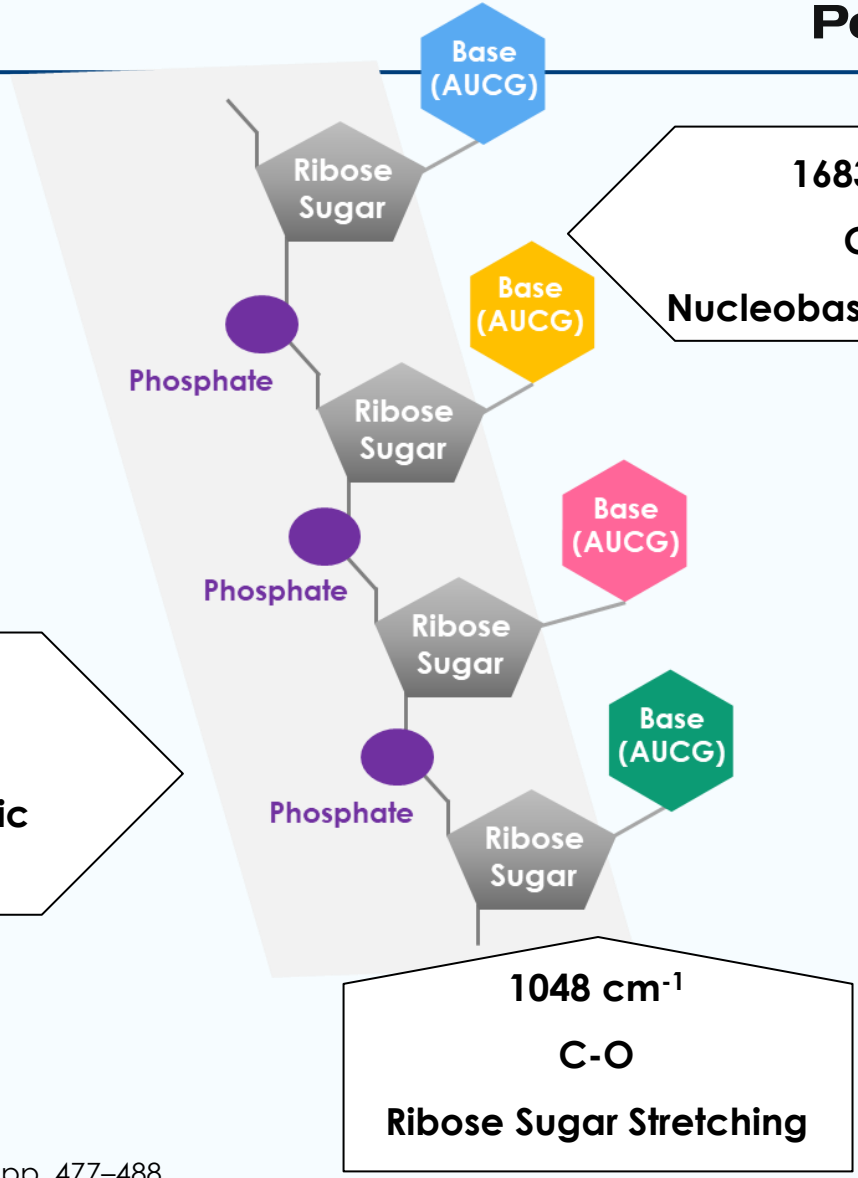
Freezing – 20°C

ATR-FTIR

RNA FTIR-ATR



1215 cm^{-1}
Phosphate
Antisymmetric
Stretching



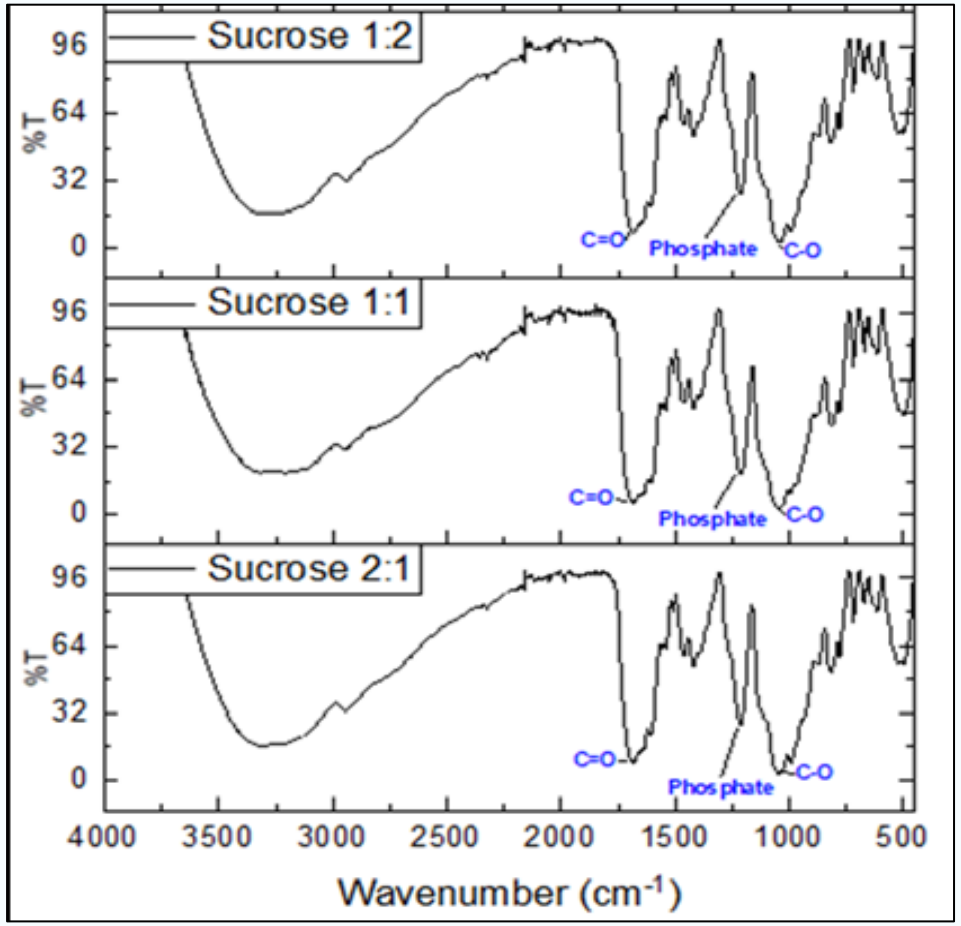
1683 cm^{-1}
C=O
Nucleobases Stretching

1048 cm^{-1}
C-O
Ribose Sugar Stretching

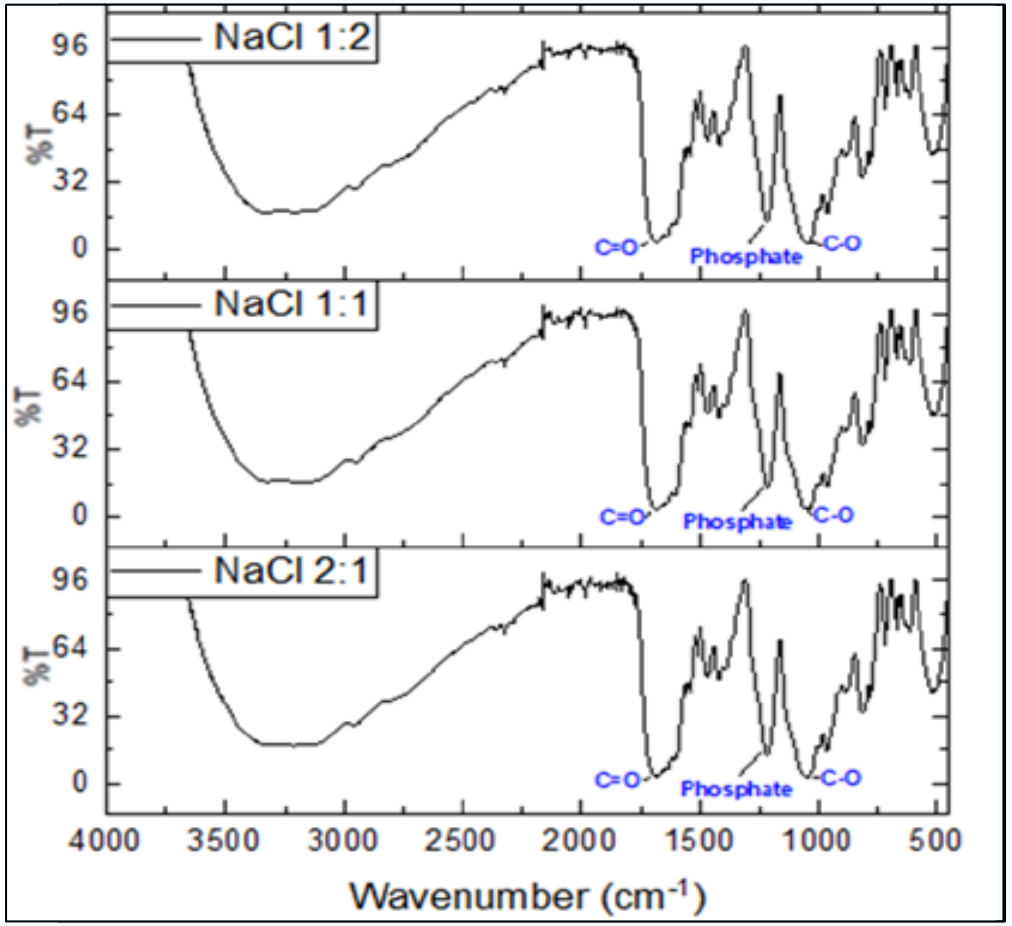
Banyay, M *et al.* (2003) 'A library of IR bands of nucleic acids in solution', Biophysical Chemistry, 104(2), pp. 477–488.
Geinguenaud, F., *et al.* (2020) 'Application of FTIR Spectroscopy to Analyze RNA Structure', in RNA Spectroscopy: Methods and Protocols. New York, NY: Springer US, pp. 119–133.

FTIR – Sucrose and NaCl Effect

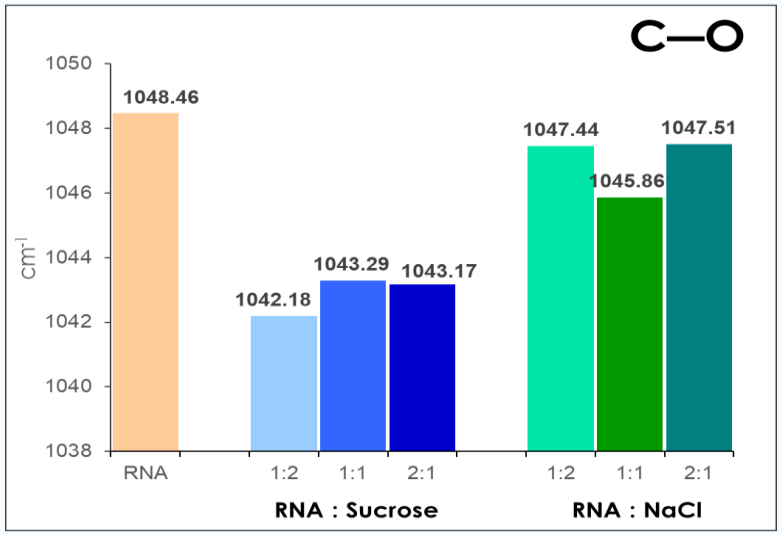
Sucrose



NaCl



FTIR – Sucrose and NaCl Effect

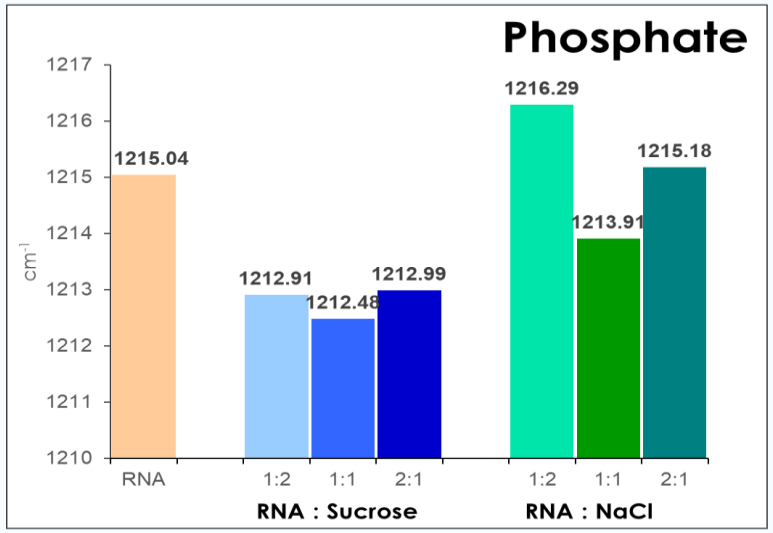


Sucrose

- Shift to lower wavenumber (5 cm^{-1})
- **Interactions** between sucrose and ribose
- Overlapping signal from sucrose hydroxyl may interfere.

NaCl

- Smaller shifts to lower wavenumber
- **No trend** with RNA: NaCl ratio.

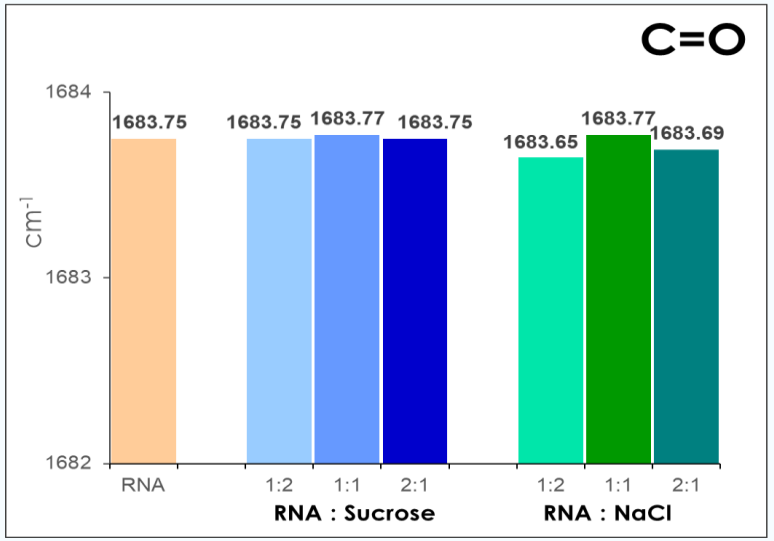


Sucrose

- Shift to lower wavenumber (3 cm^{-1})
- No trend with RNA: sucrose ratio
- **Interactions** between the **phosphate** of the RNA backbone and **sucrose**

NaCl

- Higher or lower wavenumber shift
- **Interaction** between **NaCl** and negatively charged **phosphate** of the RNA backbone
- **[Na^+] ion effect**



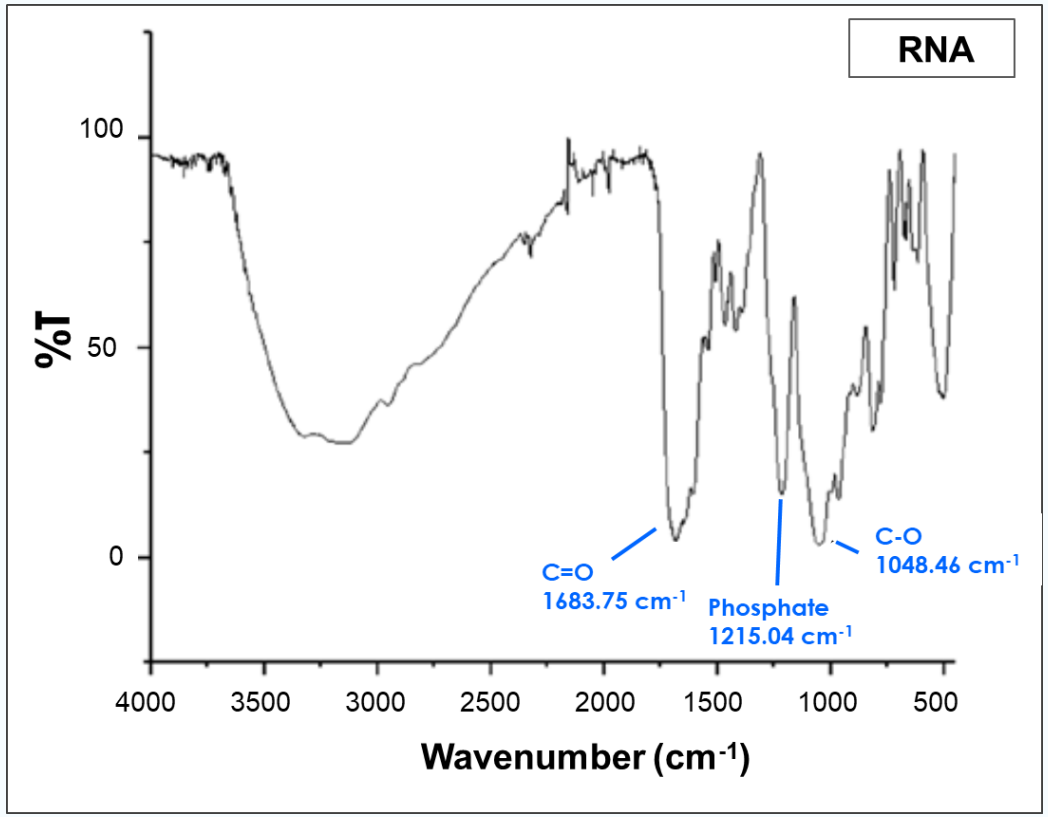
Sucrose

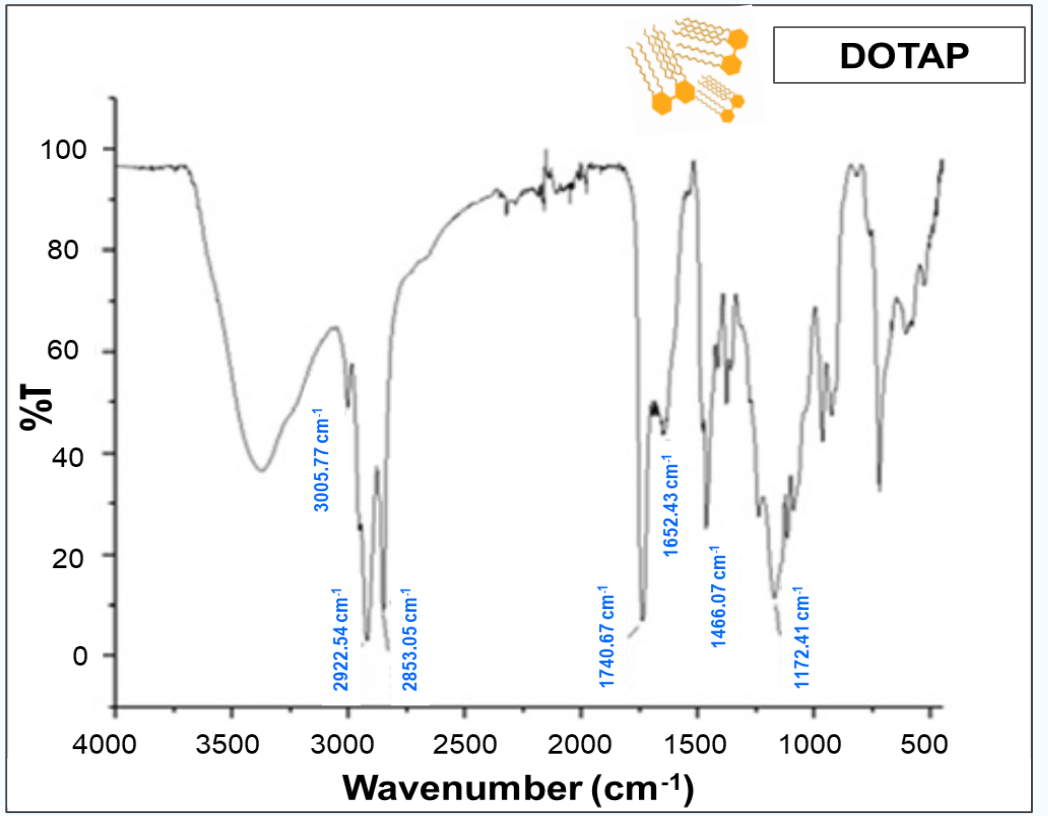
- No shift
- **No interactions** between the nucleobase carbonyl groups and sucrose

NaCl

- No shift
- **No interactions** between the nucleobase carbonyl groups and NaCl

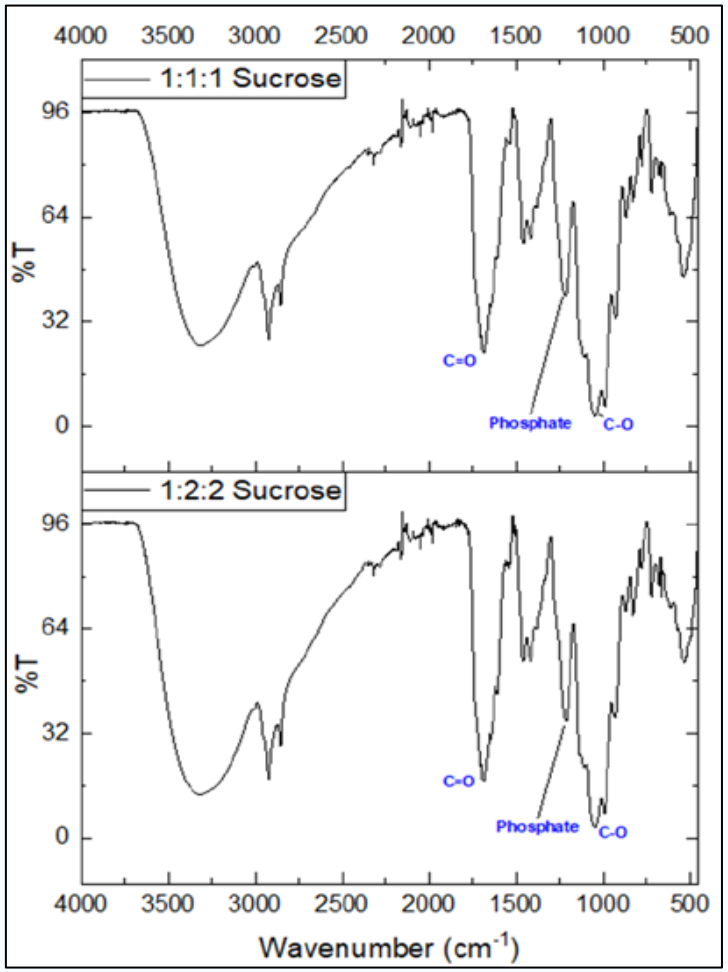
DOTAP – Sucrose and NaCl Effect



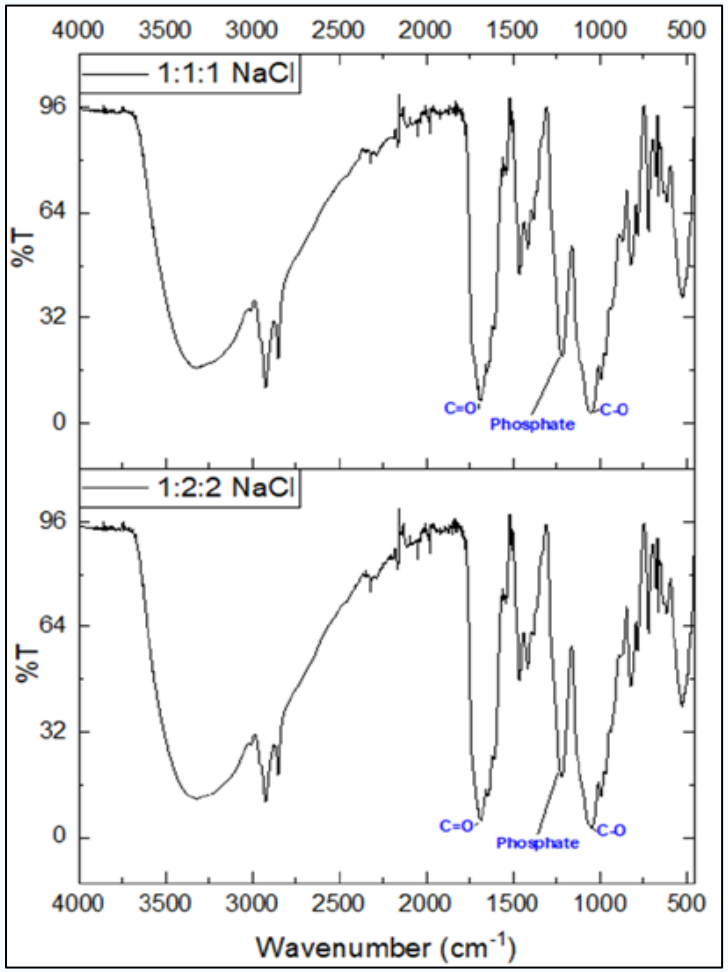


DOTAP Cationic Lipid Effect

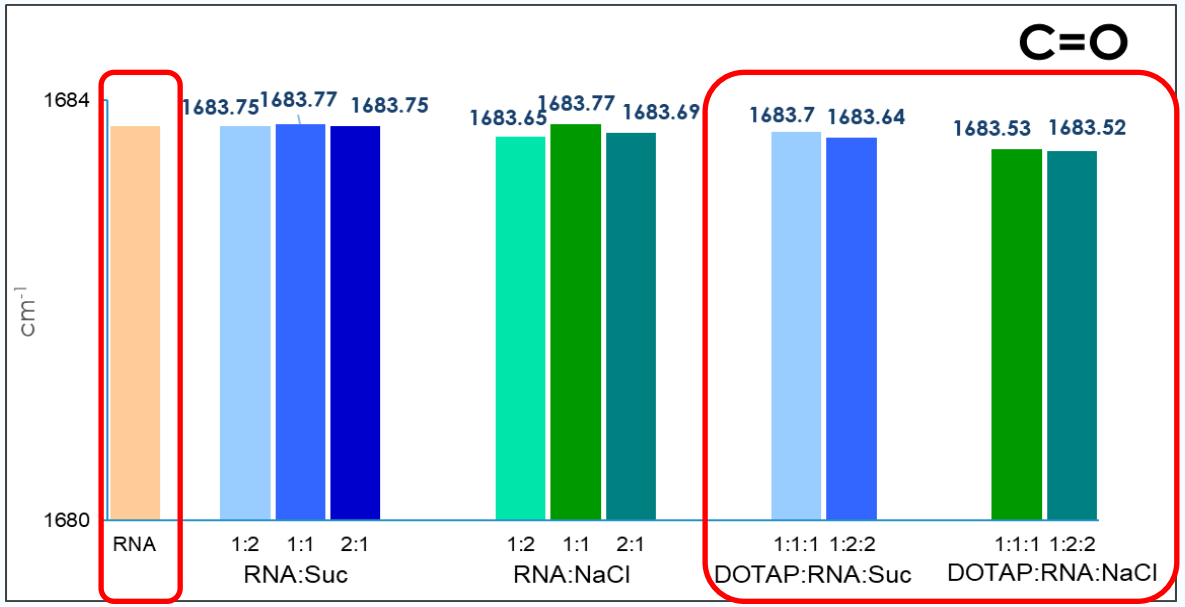
DOTAP: RNA : Sucrose

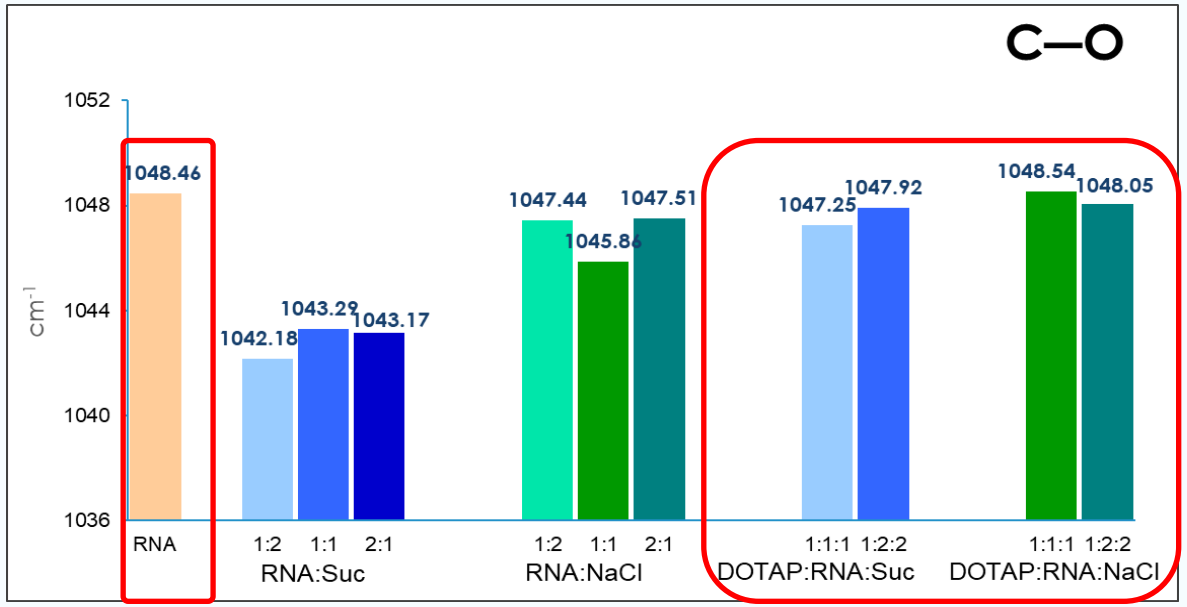


DOTAP : RNA : NaCl



DOTAP Cationic Lipid Effect

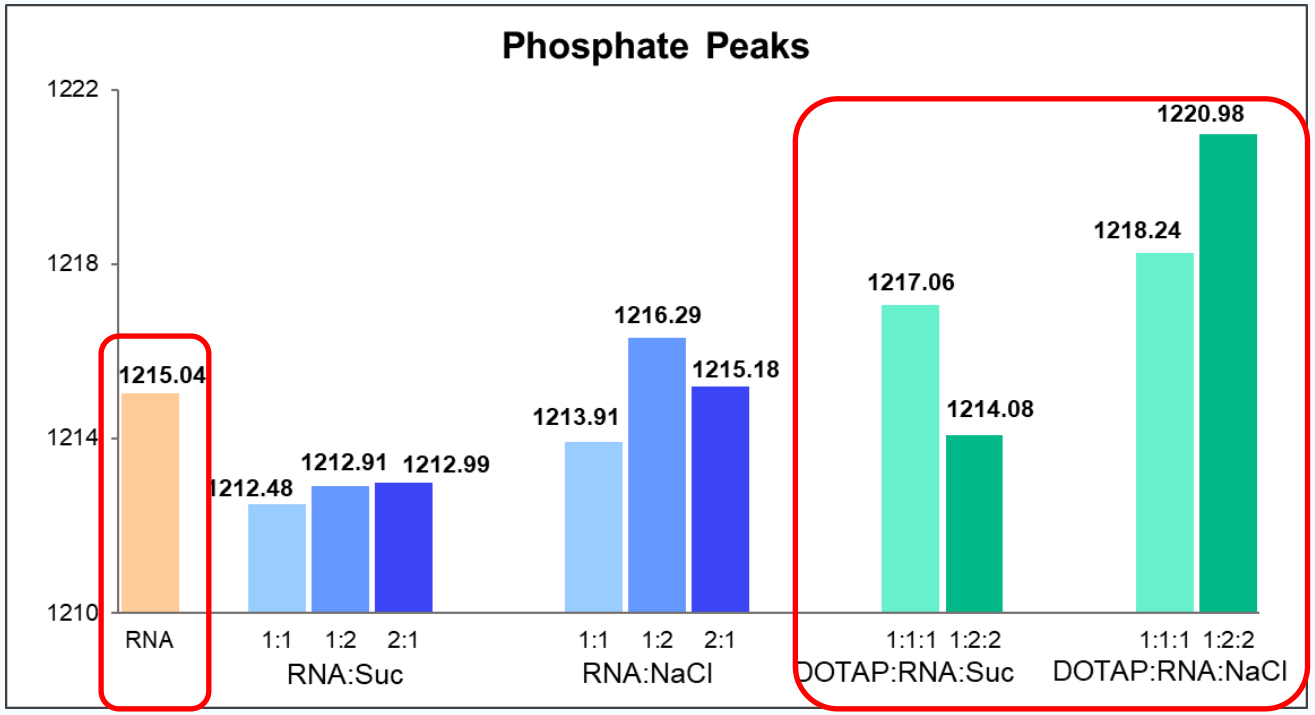


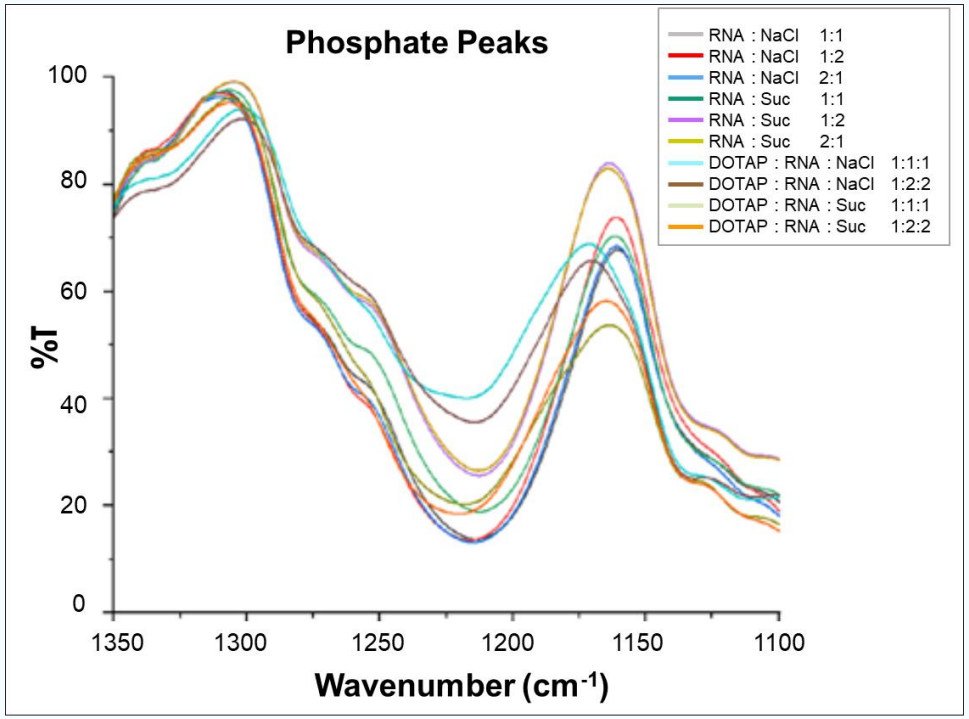


C-O and C=O band positions were essentially unchanged from RNA lyophilized without excipient for both sucrose and NaCl containing solids.

NO Interactions

DOTAP Cationic Lipid Effect



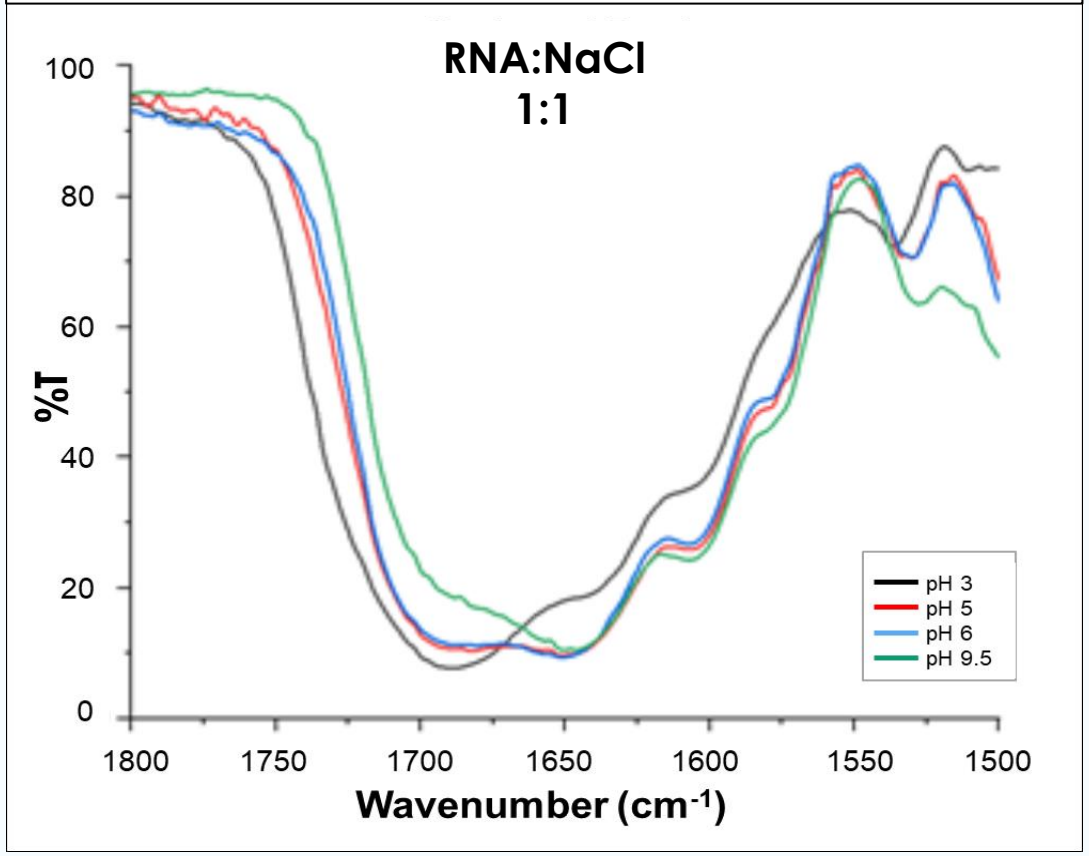


- Higher wavenumbers shift
- Trend with excipient type
- Trend with DOTAP: RNA: excipient ratios

Interactions between **DOTAP** and **RNA** via **phosphate groups** even in the presence of ionizable excipient NaCl.

DOTAP Cationic Lipid Effect – pH Effect

Carbonyl Group Band
pH Effect

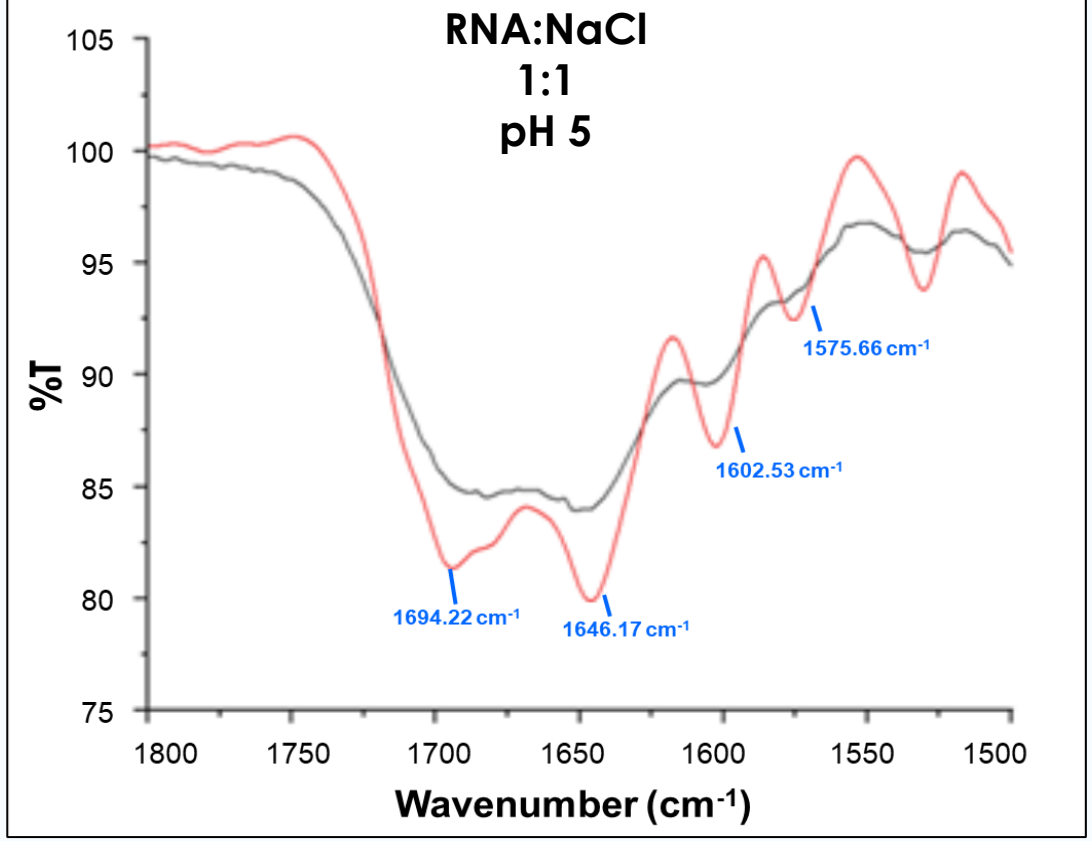


- No shift in the carbonyl band on addition of DOTAP
- Pre-lyophilized **pH effect** on carbonyl band splitting
- **pH 3** the main band appears at **1689 cm^{-1}**
- **pH 5** band splits into two peaks **1682 cm^{-1}** and **1651 cm^{-1}**
- **pH 6 - 9.5** shorter wavenumber shift **1650 cm^{-1}**

Different Conformations or **Different Environments** for the **Nitrogenous Bases**

DOTAP Cationic Lipid Effect – pH Effect

Deconvolution Carbonyl Band



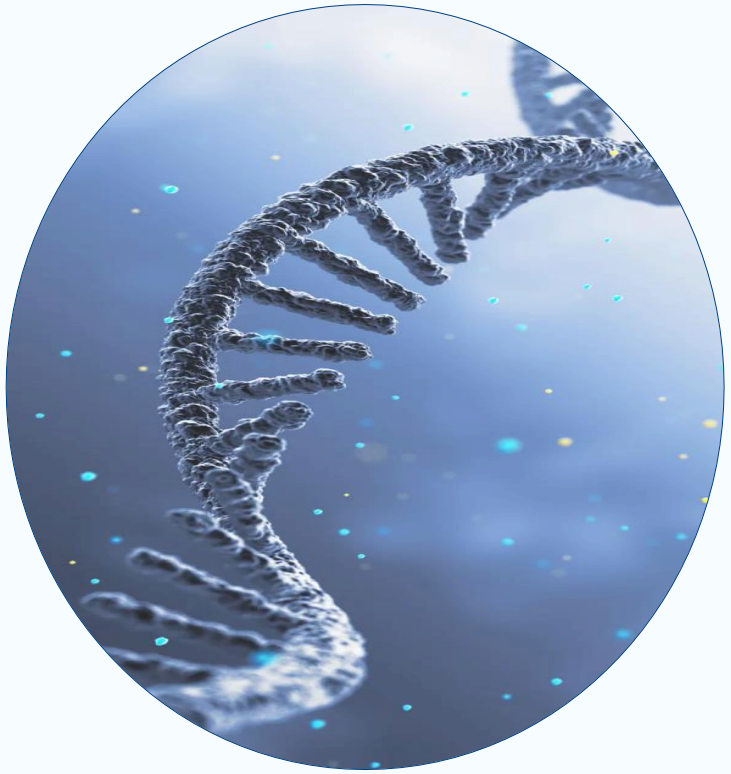
Deconvolution with gamma value of 5 and Bessel smoothing filter with smoothing length of 85% to reduce noise

Four peaks were resolved

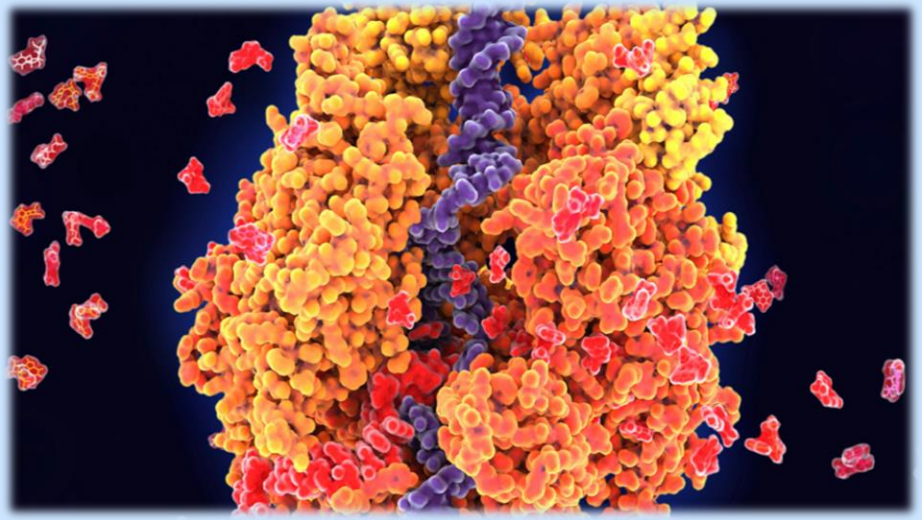
1694 cm^{-1}
1646 cm^{-1}
1602 cm^{-1}
1575 cm^{-1}

C=O in the **different nucleobases** or in **different solid environments**

Conclusion



- mRNA is a large biomolecule that needs to be formulated within specialized nanoparticles, to avoid degradation and to facilitate its transit across cell membranes
- ATR –FTIR Spectroscopy is a fast, simple and sensitive technique to characterize mRNA formulations and excipients compatibility
- IR spectra of the formulations studied show that ionizable excipients interact with the negatively charged phosphate group of RNA
- Important first step toward the understanding of mRNA stability as phosphodiester bond cleavage plays a key role in the chemical degradation of mRNA



Special Thank You

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THANK YOU FOR LISTENING!

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FOR MORE INFO

SCAN ME