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Graphene impurity in biotechnolgical products: Particle size, idrophilic properties and technological implications: HYPOTESYS OF WORK

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Abstract: Based on the fact that if present graphene in vials of nanolipids of biopharmaceutical the aspects must to be coloured the reported findings by some independet researcher of graphene like particle in viasl of m Rna covid-19 vaccine seem to suggest that this impurity can be present only inside the nanolipids. The production fo this encapulated biotecnological product imply the mixing of an acqueos phases (mRna) with an lipidic phases (nanolipids). The graphene finded as reported, probably due by purification steps, because idrofilic it must to be encapsulated. The Raman spectra of registrated m RNA C19 vaccine not revealed graphene particle , but in literature it is reported that the direct tecchnique is no the best way to to detect well the nanolipids payload : so a negative results not imply this it not present inside the nanoparticle. Aim of this work is to investigate the manifacturing process of this biotechnological products submitting to the reseacrher an hypotesys of work than can explain all this pheneomena: the possible segregation of graphene inside the nanolipids. Great enphasys is given to the size , the chemico physical property like idrophilicity of the graphene particle versus the nanolipids used in some m RNA C19 vaccine, and the property of inside /outside this particles.

Keywords: material science, graphene, size, idrophilic, manifacturing, nanolipids, segregation, chemico physical property, Purification, impurity, raman spectra, peaks, interference, nanolipids, payload.

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INTRODUCTION

In order to start this research it is of interest to introduce some concepts related physical properties of materials like the SIZE and idophilic – idophobic charcateristics:

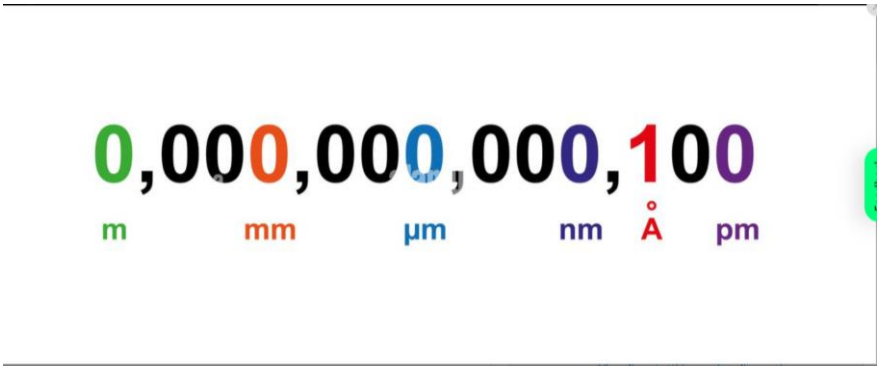


Fig n1 Size measure of particles

About the size it is possible to verify that : Particle Graphene NANOPLATELETS Dimension 25 µm SIGMA ALDRIC CAS:7782-42-5

Form https://www.ottokemi.com/graphene/graphene-98-gr-522.aspx

Graphene SIize >50 µm CAS 1034343-98-0

Novoselov and Geim in 2004 where an adhesive tape was used to detach graphene from graphite crystals. From http://www.graphenesq.com/whatis/how.asp

"This year's winners of the physics prize, Andre Geim and K.Novoselov, from Manchester University, UK, extracted graphene from the common material known as graphite - widely used as the "lead" in pencils." IN 2010 Nobel price for Physics

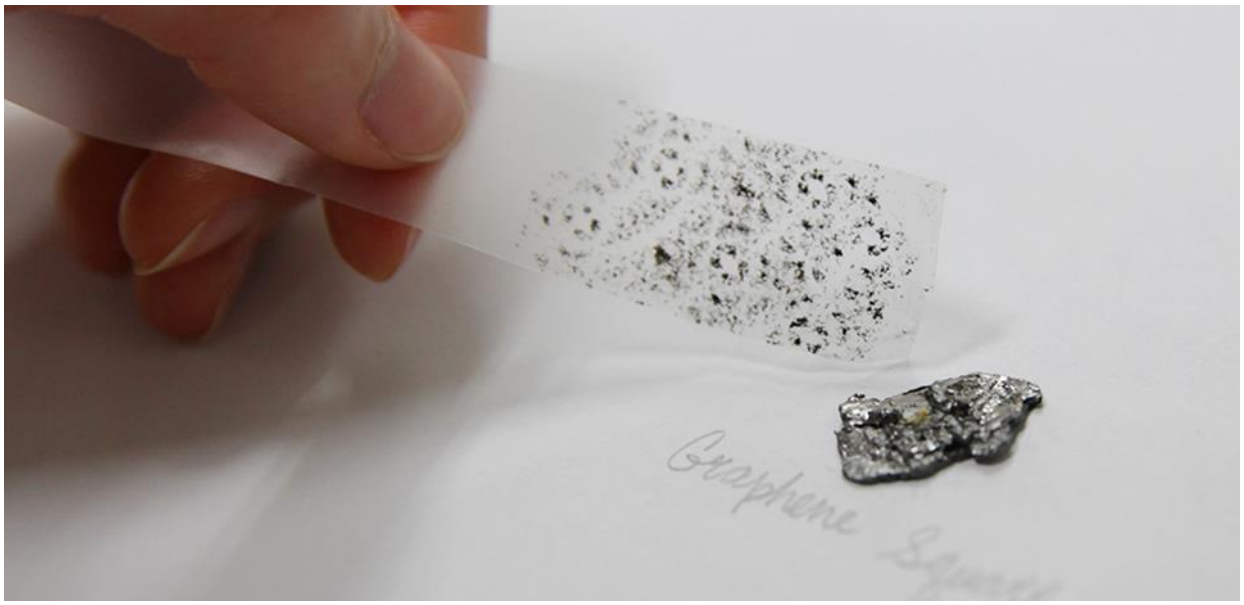


Fig . n 2 from http://www.graphenesq.com/whatis/how.asp

Journal of Science: Advanced Materials and Devices Dec 2017

Effects of initial graphite particle size and shape on oxidation time in graphene oxide prepared by Hummers' method Seyyedeh Saadat Shojaeenezhad, M. Farbod, Iraj Kazeminezhad "The average particle size of the first, second , third samples were 18, 6, and 25 µm, respectively."(1)

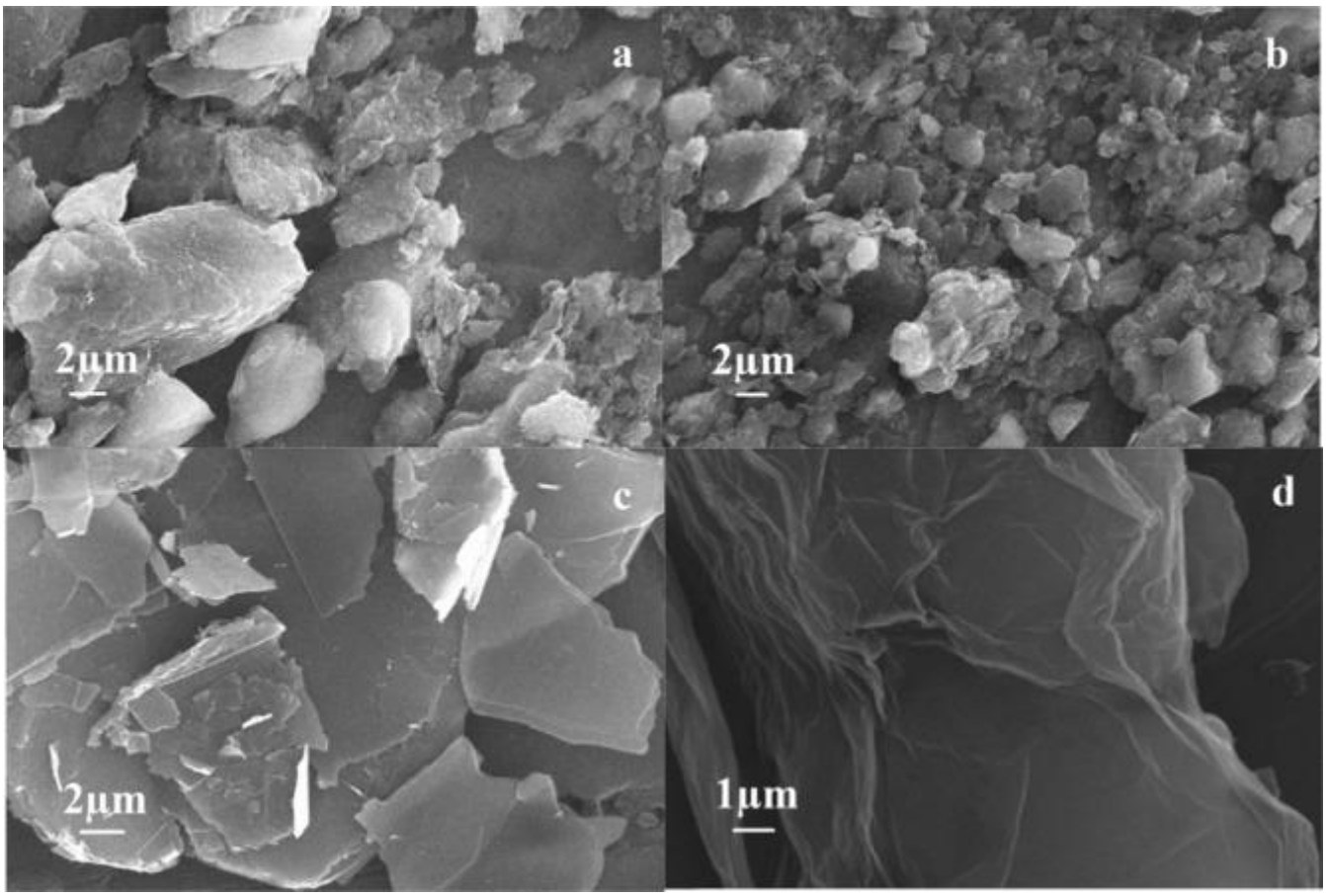


Fig n 3 Fig. 1. SEM images of the initial graphite a) the unmilled sample, b) the 5 h milled sample, c) the flake sample, and d) graphene oxide. From ref n 1

From January 10, 2022 by Michael Ducharme

Dr Andreas Noack's Warning to the World about Covid Jabs. “These graphene structures (AKA monolayer carbon or monolayer graphite) are so stable, every chemist knows this. They are not degradable. **The structure is 50 nm long and 0.1 nm thick”**

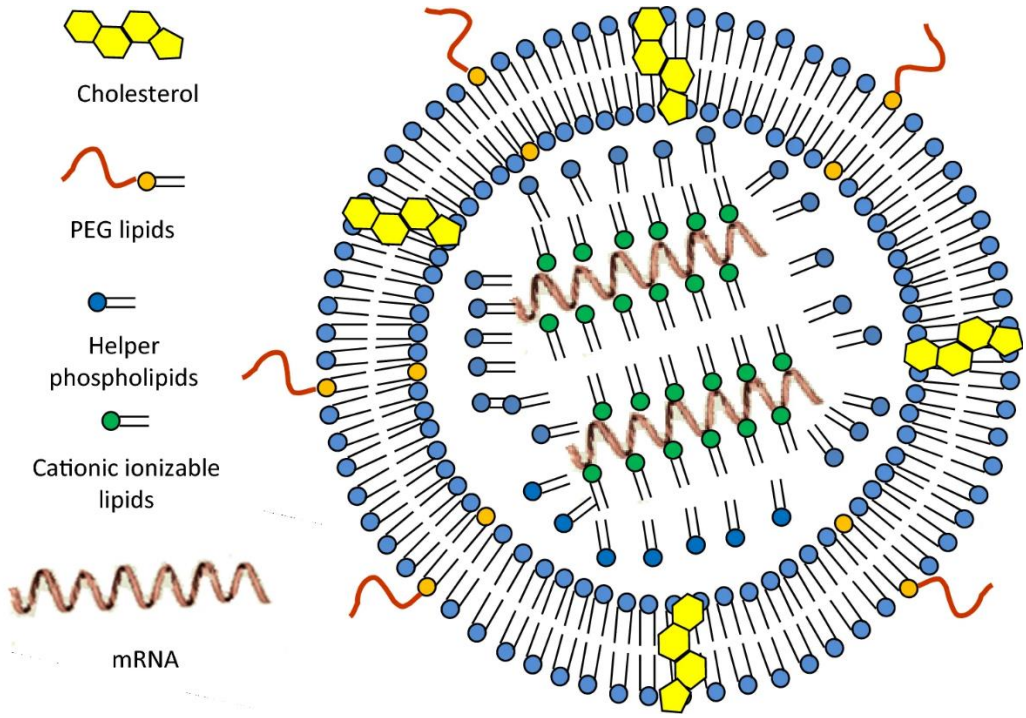


Fig n 4 Schematic representation of components of lipid nanoparticles (LNPs). PEG polyethylene glycol From doi.org/10.1007/s40290-021-00417-5

According Meenu Mehta et al "Liposomes can be synthesized into unilamellar or multilamellar vesicles, with sizes varying from 20 to 1000 nm and Solid lipid nanoparticles were initially formulated as small spherical particles with a solid lipid core at room temperature, and subsequent advancements have led to the development of flat ellipsoidal or disc-like shapes, exhibiting sizes between 50 and 100 nm. "

Sci Rep. 2023; doi: 10.1038/s41598-023-42274-z

Quantitative size-resolved characterization of mRNA nanoparticles by in-line coupling of asymmetrical-flow field-flow fractionation with small angle X-ray scattering. Melissa A. Graewert et al.

"The success of mRNA nanoparticles for vaccination against Covid-19 has highlighted the potential of RNA nanomedicines as well as of nano-scaled pharmaceutical products in general. In mRNA vaccines, so-named lipid nanoparticles (LNPs), which are characterized by a specific lipid composition and manufacturing process, are used for mRNA delivery. There is a wealth of different other nano-scaled pharmaceuticals which have reached various stages of clinical and preclinical development.

The particles may be based on organic (lipids, polymers, polypeptides, proteins) or inorganic materials (metals, metal oxides, silica).

The majority of these products are intended for parenteral application with particle sizes typically below 200 nm (the limit for the sterile filtration): LNPs measure 100 nm or less, and certain particle formats (for targeting tumors or crossing the blood–brain barrier BBB) are in the range of tens of nanometers. With dimensions of a few or few tens of Angstroms, other types of drug formats like as biologics (therapeutic proteins, antibodies) or soluble polymers may be included for this category of nano-sized drugs. The characteristics of these systems are dominated by their colloidal nature, where particle size and size-related attributes are of fundamental importance for quality, biological efficacy, and safety." (2)

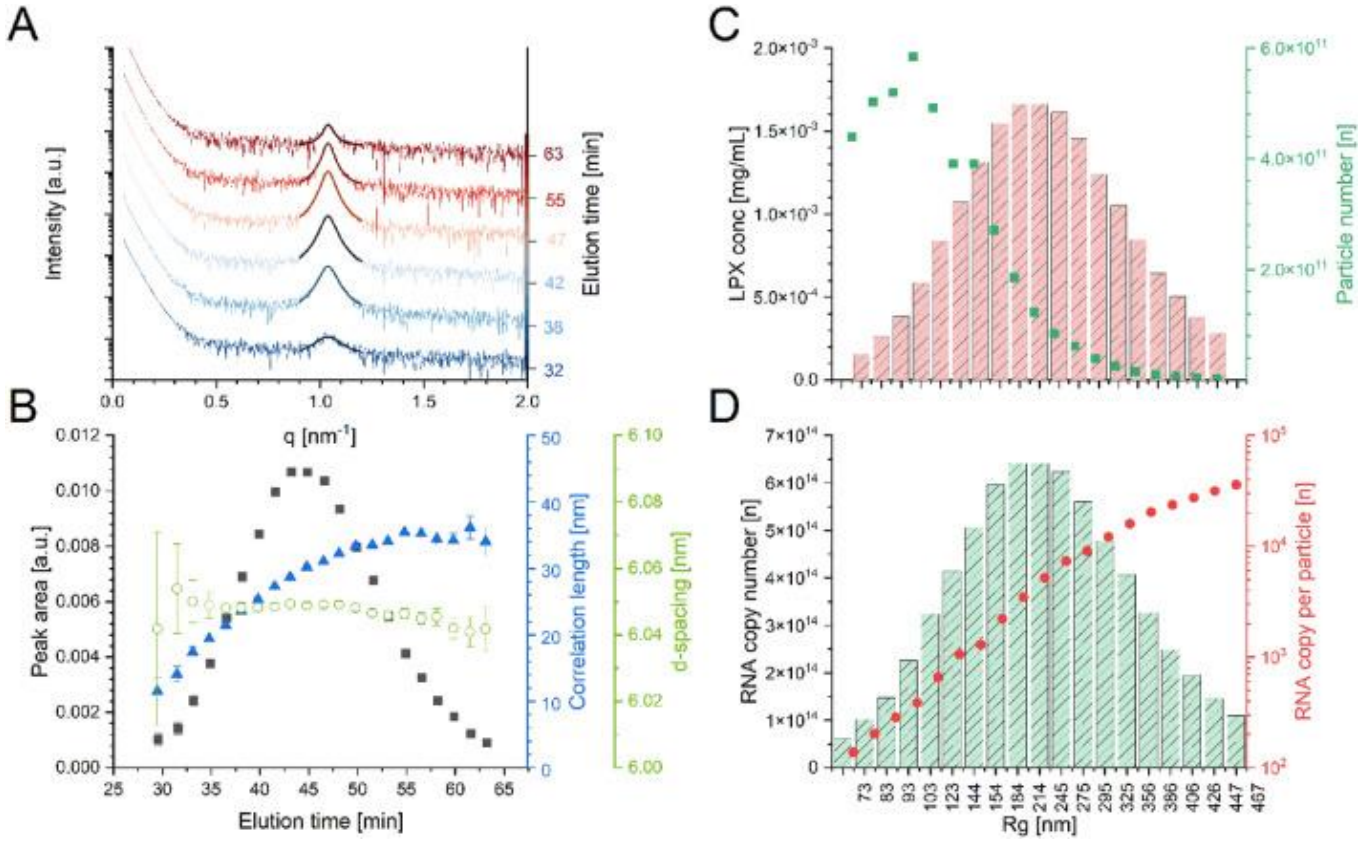


Fig. n 5 (C) Absolute material concentration (LPX conc. in mg/mL) derived from SAXS signal for LPX peak (red bars) and the calculated absolute n. of particles as a function of radius of gyration (Rg) derived from MALS (green dots). From doi: 10.1038/s41598-023-42274-z

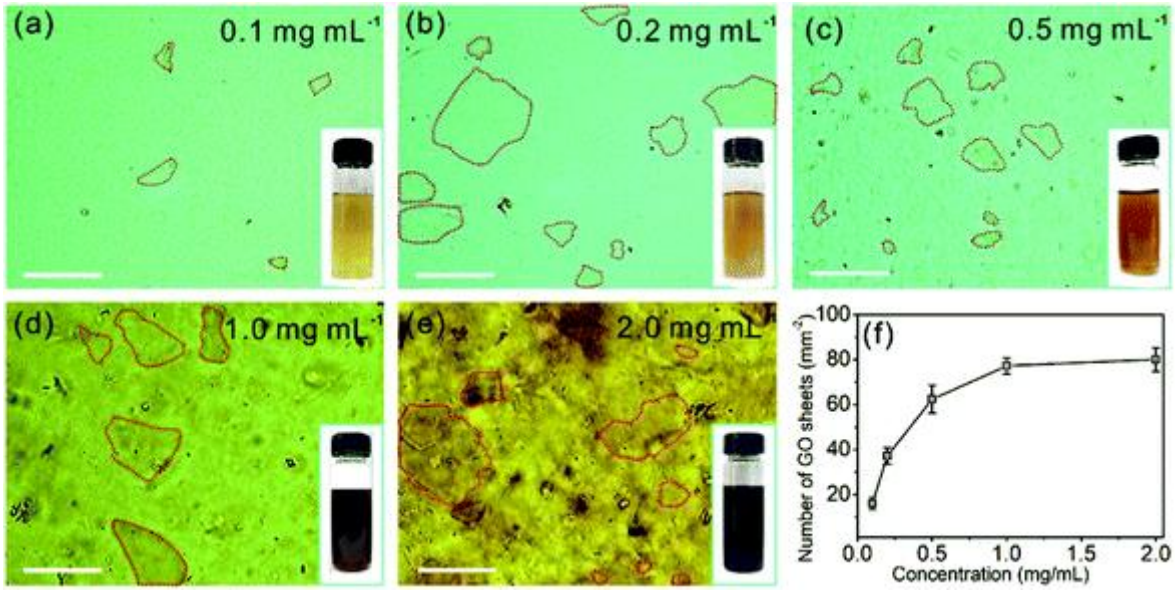
(200 Nanometer = 0, 2 micrometer)

In PREPRINT REVIEW ON ANALYTICAL METHODS FOR THE CHARACTERIZATION OF GRAPHENE STRUCTURES AND TOXICITY PROFILES. Amedeo Cinosi , L. Bolgan and Carlo Martelli.

Is reported:

"the potential spectral contributions from nanostructures within the matrix, such as graphene sheets. 10 exponent – 2 (0,01 micrometer) and 10 exponent- 3 µm (0,0001 micrometer), are masked by other compounds in the matrix." And " The structures observed in the P. vaccine by TEM are described by Young as clusters/aggregates of graphene, but both the morphological characteristics and t**he dimensions (50 µm**) are not correlated with graphene phases known from the literature or studied in a clinical context. The presence of suspended solid particulate in a colorless liquid (water or organic solvent) imparts a characteristic color, according to Lambert-Beer's law. Iron imparts a red color, and the intensity is proportional to the concentration and the path of light through the medium. The presence of even a few parts per million (ppm =

µg/ml) of graphene nanoparticles in the preparations should give a straw-yellow coloration. This is not observed in the doses of sera analyzed, as they appear colorless or milky-white;" (3) (0,01 micrometer = 10 nanometer)



Fig, n 6 a)–(e) OM images of GO suspensions in water at conc. in the range of 0.1 to 2.0 mg mL−1. The GO sheets are marked by red dashed lines. The insets in images (a)–(e) show photographs of the GO suspensions in 20 mL vials. Scale bars: 100 µm. (f) The number of GO sheets calculated by counting the GO sheets in the OM images as a function of the GO concentrations. From https://doi.org/10.1039/C9RA02076D

Some independent researcher finded particles similar to graphene into some vials of c19 vaccine or in blood of vaccinated with size about. (It is also to be considered to self asselbling properties of graphene particle).

It was finded 19- 114 micrometers or more size in blood of vaccinated (Ki-Yeob Jeon et al), or voluminoses particle of about 329.14µm by 137.74µm five week after vaccination (graphene particles?) Benzi Cipelli et al.

Form Nanotechnology, Nanomaterials AUGUST 18, 2015. Is graphene hydrophobic or hydrophilic? by National Physical Laboratory

"the findings indicate that the graphene hydrophobicity is strongly thickness-dependent, **with single-layer graphene being significantly more hydrophilic than its thicker counterparts."**

And in Dissertation: Understanding the intrinsic water wettability of graphite. Kozbial, Andrew (2016) Understanding the intrinsic water wettability of graphite. Doctoral Dissertation, University of Pittsburgh.

"This work unequivocally shows that fresh graphitic surfaces are mildly hydrophilic." J Chem Phys . 2015 Oct 21; doi: 10.1063/1.4933011.

Hydrophilic behavior of graphene and graphene-based materials. Sebastián R Accordino , Joan M. Montes de Oca , J Ariel Rodriguez Fris , Gustavo A Appignanesi.

"Our molecular dynamics studies will demonstrate that parallel graphene sheets present strong tendency to remain fully hydrated for a moderately long times (even when the equilibrium state is indeed the collapse of the plates), they are less prone to self-assembly than the model hydrophobic surfaces we shall employ as control which readily undergo a hydrophobic collapse. Potential of mean force calculations will indeed make evident that the solvent exerts a repulsive contribution on the self-assembly of graphene surfaces"

Materials and methods

With an observational point of view various concepts of phisycal chemistry relates Size and idorphilic properties are reported. Relevant literature is analysed and figure reported help in clarify the concepts related the hypotesys Submitted. An experimental project hypotesys is provided to test this process. Finally a global conclusion make possible to resume all

Results

Form literature

Lee, Y. M. et al (2022). Foreign materials in blood samples of recipientsof COVID-19 vaccines. International Journal of Vaccine Theory, Practice, and Research, 2(1), 249–265.

"they also found components that the CDC had claimed were not used — including reduced graphene oxide (rGO), or graphene hydroxide (GH), in the M. vaccine.

The diameter they observed at 100µm was apparently the same as the one we have pictured above in Figure reported . We also showed a slightly smaller very similar structure ."(4)

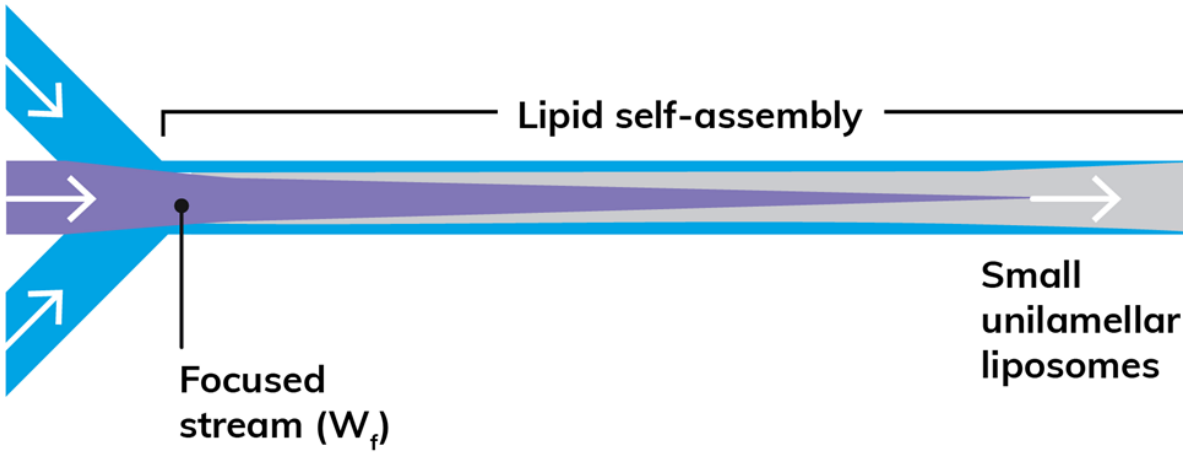


Fig n 7 from https://www.ondrugdelivery.com/automated-lipid-nanoparticle-production-from-protocol-development-to-gmp-manufacture/

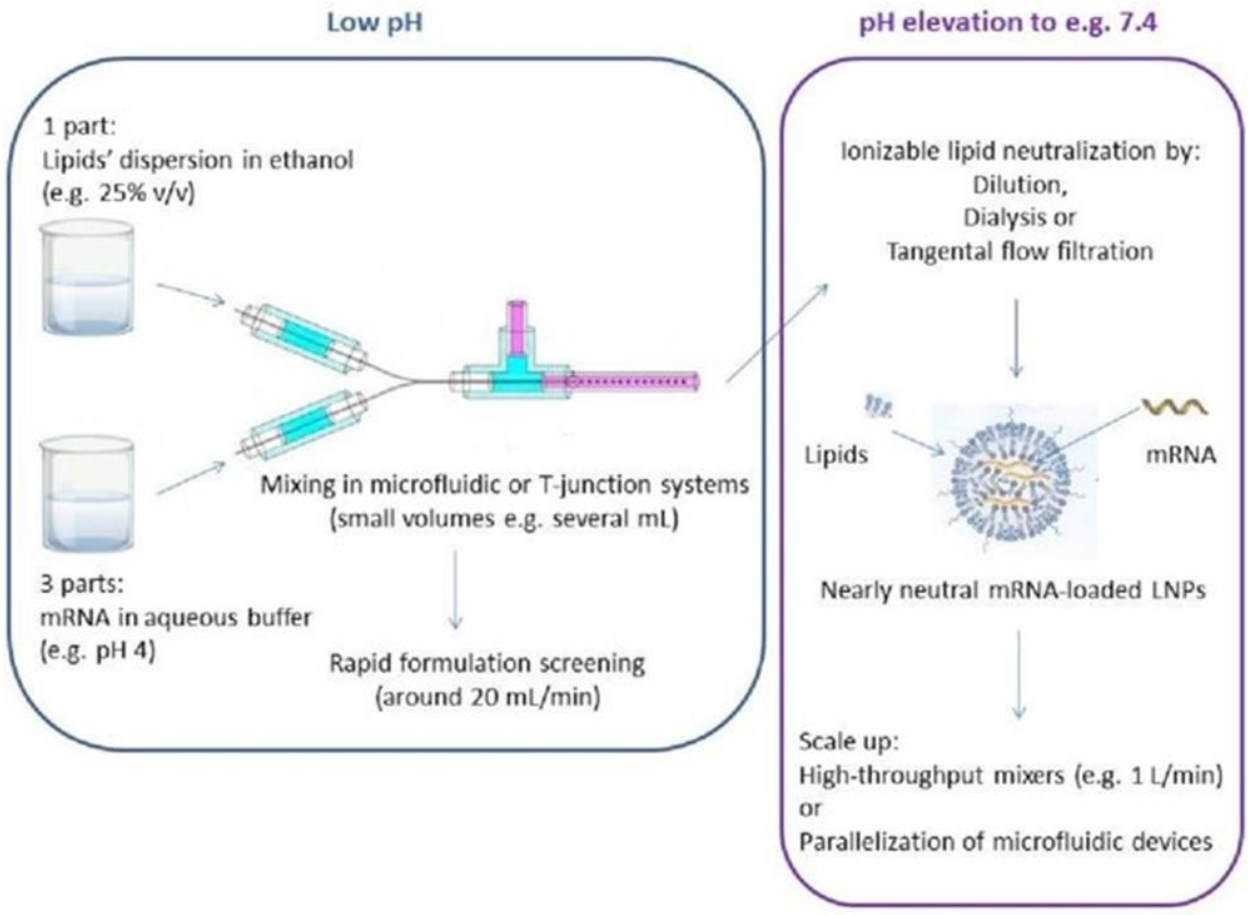


Fig. n 8 Schematic presentation of a manufacturing approach to obtaining mRNA encapsulated lipid nanoparticles as potent vaccines for COVID-19 prevention. From Lipid nanoparticles employed in mRNA-based COVID-19 vaccines: An overview of materials and processes used for development and production

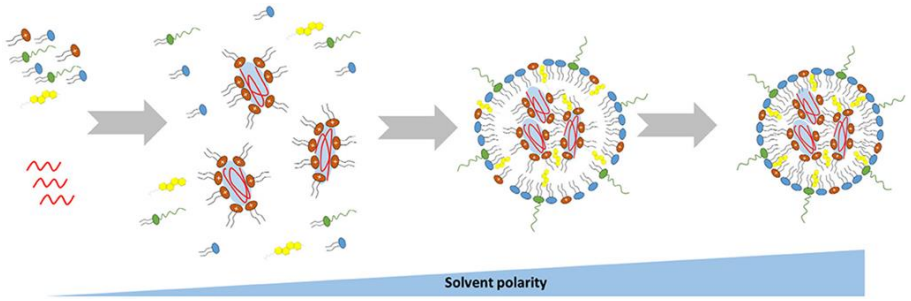


Fig. n 9 Schematic representation of the mechanism driving the self-assembly of mRNA-loaded lipid-based nanoparticles

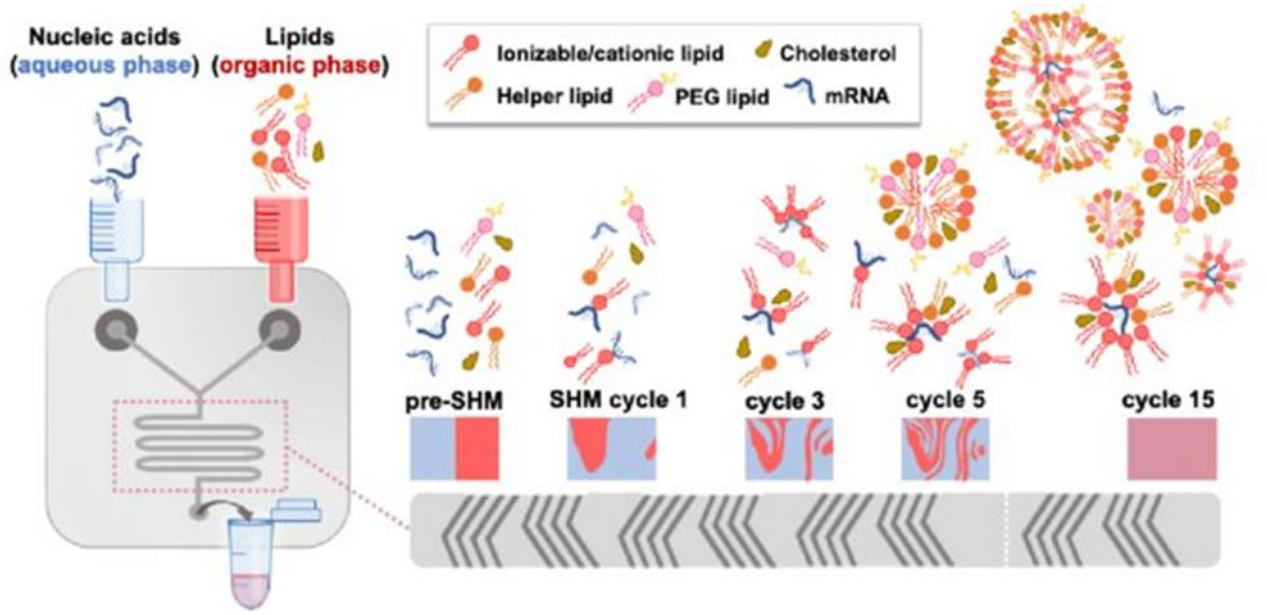


Fig. n 10 Lipid nano-particle formation utilizing a microfluidic platform with a staggered herringbone micromixer . Within the microfluidic channels, the SHM allows the aqueous phase (containing the nucleic acids, mRNA, under an acidic pH) and the water-miscible organic phase (containing the lipids and cholesterol) to proceed from laminar flow (pre-SHM) through several cycles of chaotic mixing until complete mixing of the phases has occurred (cycle 15).From Emily Pilkingtonab et al

From Is Graphene Hydrophilic or Hydrophobic? Posted by MSE Supplies Admin on May 11, 2020

"Materials scientist in the Netherlands furthered this study work by measuring the contact angle of water on the graphene and several other kind of materials. They found that with water the graphene surface was smooth and clean meaning the graphene material was indeed hydrophilic"

PREPRINT REVIEW ON ANALYTICAL METHODS FOR THE CHARACTERIZATION OF GRAPHENE STRUCTURES AND TOXICITY PROFILES. Amedeo Cinosi , Loretta Bolgan and C. Martelli.,

"The presence of even a few ppm (ppm = µg/ml) of graphene nanoparticles in the preparations should give a straw-yellow coloration. This is not observed in the doses of sera analyzed, as they appear colorless or milky-white"

Regulatory agency allow use of direct (non sample destructive) RAMAN spettroscopy in PAT : but according literature this method without pretreat the sample with solvent is not adeguate to measure nanolipids payload. (5)

Anal Bioanal Chem. 2022 2021 Oct 20. doi: 10.1007/s00216-021-03727-4. The role of Raman spectroscopy in biopharmaceuticals from development to manufacturing. Karen A. Esmonde- et al

"Raman spectroscopy RS has been used since 2006 to provide analytical -quality control of compounded formulations stored in vials or directly through polymeric infusion pumps in hospital setting" (6)

In the light of this recent investigation, does the Commission intend to have an independent analytical chemical laboratory perform a careful analysis to check for the presence of graphene in the C-19 vaccines?" Last updated: 27 Jan 2022 Parliamentary question - P-000303/2022(ASW)

European Parliament. Answer given by Ms Kyriakides on behalf of the European Commission. 8.3.2022

Written question

"EMA has **analysed reports** describing the analysis of several vials of C-19 vaccines suggesting the presence of graphene and concluded that the currently available data do not show presence of graphene in the vaccines concerned. The analysis by EMA's working party for biological medicines included an input on the

Raman- spectroscopy RS from the European Directorate for Quality of Medicines and the independent national testing lab responsible for the batch release (OMCLs). Quality control testing and quality assurance review, by the vaccine manufacturers and OMCLs responsible for batch release, confirm that each batch met all quality standards prior the release. “

Analytical and Bioanalytical Chemistry Raman spectroscopy as a process analytical technology for pharmaceutical manufacturing and bioprocessing 04 August 2016. “Since the 1980s, Raman spectroscopy RS has been used to study active pharmaceutical ingredients (API)” (7)

Luisetto M, Almukthar N, Edbey K, Tarro G, A.Nili B, et al. relevant article, white papers and other documents concerning the mRNA vaccine: an interesting collection useful to better understand some phenomena and to generate hypotesys.

J Forensic Sci Res. 2022; DOI: 10.29328/journal.jfsr.1001037. “In European pharmacopeia’s EP last edition it is allowed to use direct nondestructive methods “ (8) 20 January 2016, Strasbourg, France Council of Europe

Revised General Chapter on Raman Spectroscopy in the European Pharmacopoeia: inclusion of handheld devices, adaption to PAT purposes

“Hand-held instruments are now available on the market, which are suitable for identification purposes even though requiring different tolerances for the wavenumber scale verification than benchtop models.”

How Raman Spectroscopy RS is adapting to European Pharmacopoeia regulations Sponsored Content by Metrohm Middle East FZC Feb 15 2022. “Raman spectrometers, especially portable and handheld instruments, are being used predominantly for the QC of raw materials and medicines. Requiring less technical skill, instrument interfaces are easy to use. For several samples with rapid, non-destructive measurements, they also offer flexible sampling options.”

European Pharmacopoeia adopts revised Raman spectroscopy chapter may 2021. By Hannah Balfour (EP Review)13 May 2021. “Ph. Eur. Explained that Raman spectrometers RS are increasingly being deployed in the pharmaceutical environment because of the essential information they can provide via rapid, non-destructive measurements.”

Review of Existing Standards, Guides, and Practices for Raman Spectroscopy Afroditi Ntziouni https://orcid.org/0000-0001-9782-023X, James Thomson et al. Vol.76, Issue 7. https://doi.org/10.1177/0003702822109098

“According the IUPAC Gold Book, sample is a portion of material selected from a larger quantity of material, the term needs to be qualified (bulk sample and representative sample) and implies the existence of a sampling error (otherwise the correct term would be test portion, aliquot, specimen).” (9). Research article March 21, 2022

Review of Existing Standards, Guides, and Practices for Raman Spectroscopy Afroditi Ntziouni. https://orcid.org/0000-0001-9782-023X, J. Thomson et al. Volume 76, Issue 7 https://doi.org/10.1177/000370282210909

“Sampling Factors Affecting Raman Measurements

Raman spectroscopy RS is considered as a vibrational technique with **advantages such as little or no sample preparation and direct and nondestructive analysis”(10) Applications of Raman Spectroscopy in Biopharmaceutical Manufacturing: A Short Review. K. Buckley et al 2017**

“The other major, complicating factor in the use of Raman spectroscopy RS for biopharma is the fact that many of the aqueous solution samples (the cell culture media and spent bioreactor broths), and the biogenic molecules themselves, can have extremely complex compositions/ structure. “ (11) Appl Spectrosc . 2011 May;65(5):514-21. doi: 10.1366/10-06089.

Detection of trace melamine in raw materials used for protein pharmaceutical manufacturing using surface-enhanced Raman spectroscopy (SERS) with gold nanoparticles. Zai-Qing Wen et al DOI: 10.1366/10-06089

“The detection limit of 10 ppb in raw material dissolved in 30:70% water/acetonitrile is equivalent to 0.5 ppm in solid raw material. It has excellent linearity in the conc. range measured. The detection of melamine using the SERS technique is rapid (within 3 minutes), convenient, and requires no extraction procedure, offering an alternative method for screening melamine in raw materials RM at biopharmaceutical manufacture sites. “(12)

From AGILENT website : Raw Material Identification of Mrna Lipid Nanoparticle Components with the Agilent Vaya Raman Spectrometer. “Lipids are the building blocks of LNPs. Figure reported shows the overlay of Raman spectra rs and how the Vaya can easily discriminate the PEGylated, ionic, and sterol lipids from each other. Raman band assignments confirm the presence of the long hydrocarbon chains present in lipids. The band at 1,440 cm–1 is attributed to the deformation vibrations of CH2 and CH3,the band at 1,673 cm–1 is a result of the stretching vibrations of C=C present in the cholesterol. In DSPC, the band at 949 cm–1 corresponds to PO stretching. The band at 1,700 cm–1 is attributed to C=O stretching in both DSPC and DMG-PEG 2000 lipids.”

The band at 1,700 cm^{-1} is attributed to C=O stretching in both DSPC and DMG-PEG 2000 lipids.

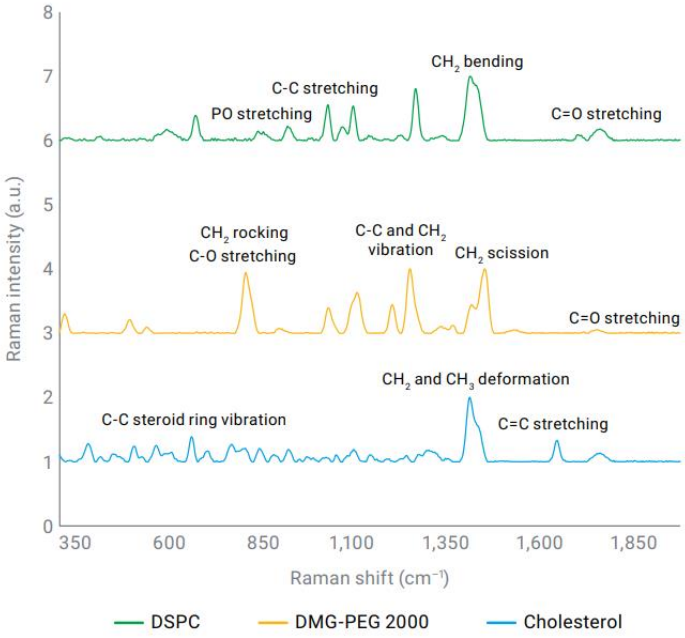


Figure 2. Agilent Vaya Raman spectra of lipids through clear glass (cholesterol, DSPC) and amber (DMG-PEG 2000) vials.

Fig n 11

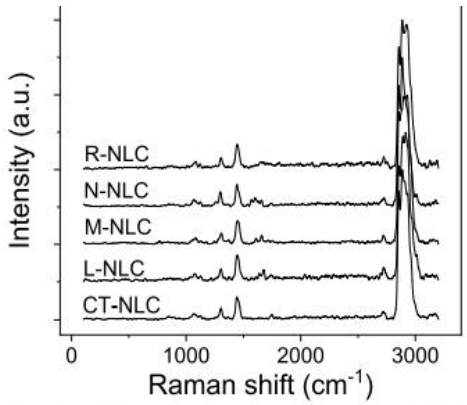


Figure 3. Raman spectra of L-NLC, CT-NLC, and N-NLC.

Fig. 12 The most intense bands in the spectra of molecules including alkanes and lipids with alkyl groups are the CH stretching modes. NLC nanostructured lipid carriers From C. CIMINO thesis

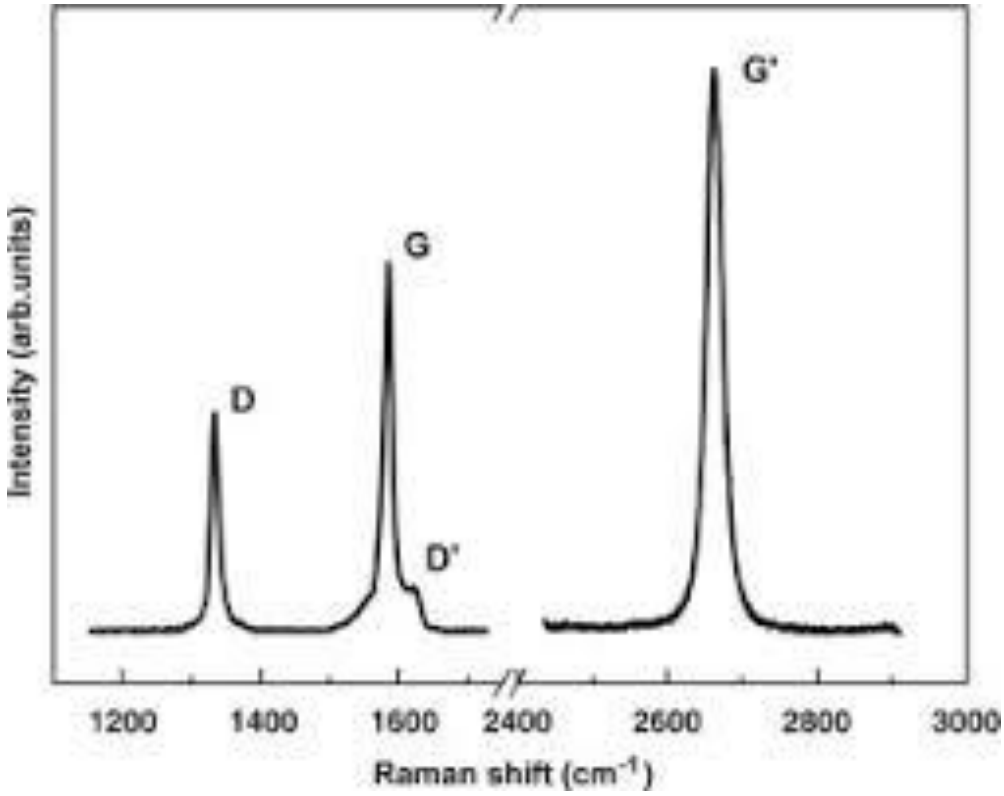


Fig. n 12 From https://doi.org/10.1016/j.physrep.2009.02.003 The most prominent features in the Raman spectra of monolayer graphene are the so-called G band appearing at 1582 cm−1 (graphite) and the G' band at about 2700 cm−1 using laser excitation at 2.41 eV

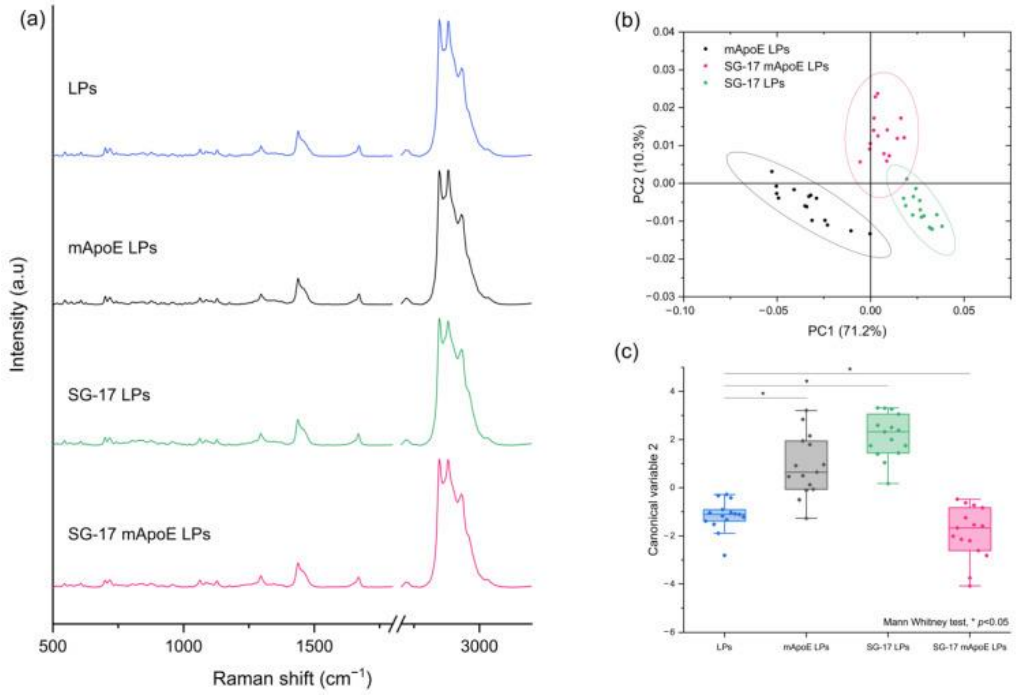


Fig n 13 form doi: 10.3390/nano13040699

Nanomaterials (Basel). 2023 Feb; doi: 10.3390/nano13040699 Raman Spectroscopy Characterization of Multi-Functionalized Liposomes as Drug-Delivery Systems for Neurological Disorders Francesca Rodà et al :"peaks at 596, 944, 1008, 1199, 1339 and 1550 cm−1" (13)

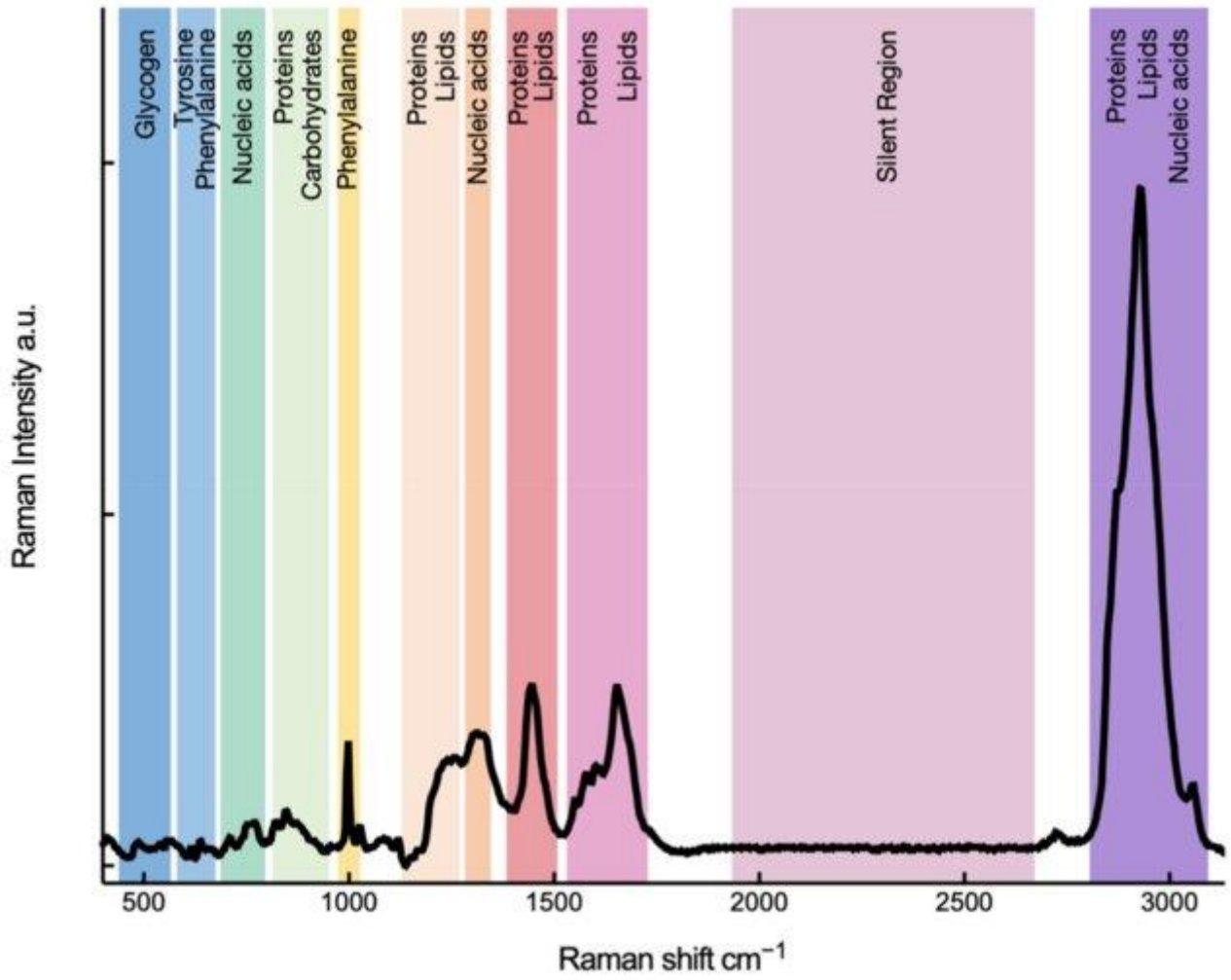


Fig. n 14 from DOI: 10.3390/cancers13071718

And related RAMAN SPECTROCOSPY: the region of fingerprint silent region and high wavenumber region:

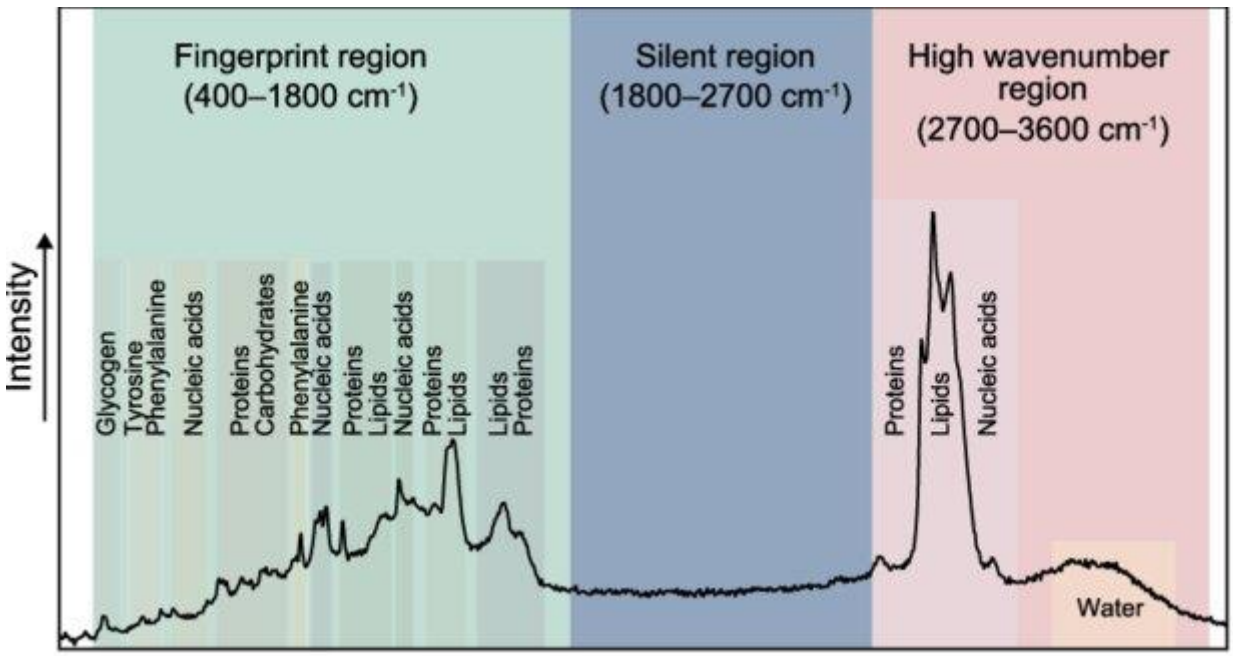


Fig. n 15 from In situ identification of environmental microorganisms with Raman spectroscopy

May 2022 Environmental Science and Eco technology 11(9):100187 DOI: 10.1016/j.ese.2022.100187

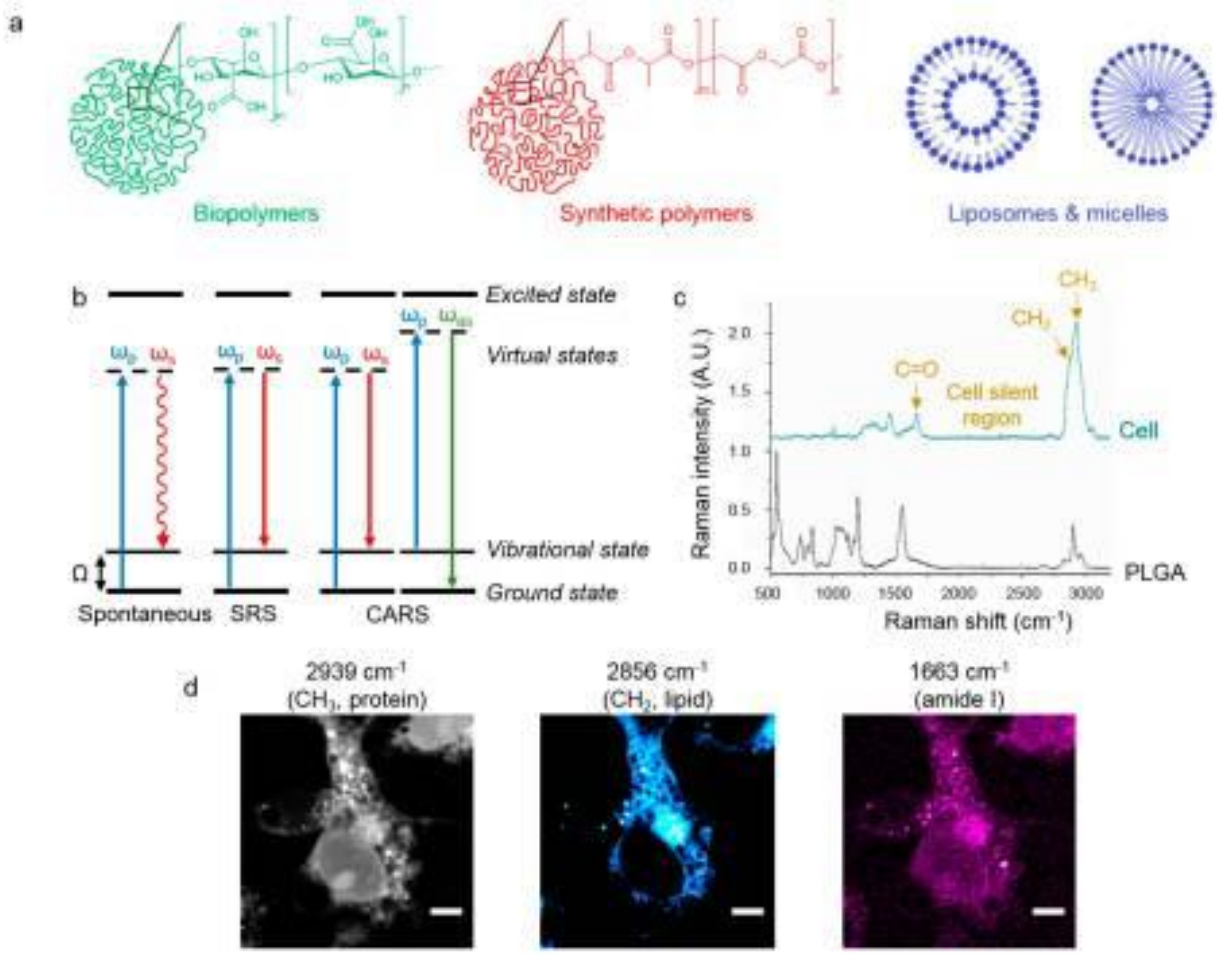


Fig. n 15 Raman imaging of nanocarriers. (a) Representation of different materials which can be fabricated into nanocarriers, such as biopolymers , synthetic polymers (PLGA), and lipids (as liposomes and micelles). (b) Energy level diagrams showing the processes of spontaneous Raman, stimulated Raman scattering , and coherent anti-Stokes Raman scattering (CARS). (c) Spontaneous Raman spectra showing the characteristic peaks in microglia (top, green spectrum) and PLGA, a common polymer for drug delivery (bottom, black spectrum). From doi: 10.3390/nano9030341

Nanomaterials (Basel). 2019. Raman Imaging of Nanocarriers for Drug Delivery, Sally Vanden-Hehir, William J. Tipping, M. Lee, Valerie G. Brunton, Anna Williams, Alison N. Hulme. "When incubated with HeLa cells, the nanoparticles were observed by Raman imaging to localize in the cytoplasm . The spontaneous Raman spectra RS show a strong, sharp alkyne peak at 2200 cm−1, along with the characteristic cellular peaks CP. Shows that the alkyne signal co-localizes with the cellular lipids (2850 cm−1) , there is an absence of signal in the cell-silent region at 2170 cm−1, confirming that the signal in the on-resonance image is due to TAT-PPE nanoparticles." (14)

Exeperimental project hypotesys Verify of segregation phenomena of graphene particle into the nanolipis.: In order to test if the hypotesys is correct is to add in artificial way to 50 Mrna sample particles of grhaphene in significative amount before to add into the lipids phase for encapsluation (group A). The control it is done without adding this artificial substantia. (group B).

After this : it must to be detected using Raman spectroscopy whit pretreating the nanolipids with solvents to make possible to avoid the matrix interference. The test will be positive, confirming the hypotesys, if graphene is finded in the group A. To complete the data registration it must to be collected the colour of the vials (group A and B)

DISCUSSION

As seen in fig n 9 From Emily Pilkingtonab et al in the manifacturing of encapsulated m RNA the acqueous phases is fisrt mixed with the lipids phases. Because Graphene is an idrophilic ,if present in the purification steps of the m RNA it must to be finded in the acquesous phases. Literature reported : NANOLIPIDIS SIZE about 200 nanometer and some GRAPHENE SHEETS SIZE also about 0,01 MICROMETER. Young finded di 50 micrometer particle, and other finded particle similar graphenic with size from 19 to 319 micrometers in blood of vaccinated.

(Graphene materials are subjected of self assembling properties.) Indepented researcher or authorities not finded abnormal coloured vials of m RNA VACCINE. The peack of raman spectra of graphene, Nanoparticles are very informative to verify the interference. The effect played by syntetic polimer PLGA nanocarrier on raman signal is clear : reduced versus the cell signal.

CONCLUSION

The chemico -physical properties of graphene particle like idrofilicity and size make possible to think that:

- the nanolipids of some biotechnological products can in theory contain graphene particles due by their specific size (in example if impuriy of the purification steps)

- the manifacturign process imply the mixing of an acqueous phase (with the m RNA) with the lipids phase

-the process is then concluded increasing the polarity of the enviroment

- if presente graphene impurity it must to be encluded into the nanolipids because its idrophilic properties.

-because encapsulated, the Raman direct technique can not adeguately detec because matrix effects

(this techniqye is not efficacy for test the payload) :see the peak of RAMAN spectra of the graphene and the Nanolipids. In this way the vials can appare not coloured? (if the graphene is only encapsulated). (to be verified) Performing the experimental project submitted is possible to test if this hypotesys is real or not. It is opinion of the authors that the process reported can explain what was finded by some independent researcher and what not was finded using Direct Raman spettroscopy by producers and regulatory Body. In some cases the reality can show different perpective depending by what approach and vision method is used.

References:

1. Shojaeenezhad, S. S., Farbod, M., & Kazeminezhad, I. (2017). Effects of initial graphite particle size and shape on oxidation time in graphene oxide prepared by Hummers' method. *Journal of Science: Advanced Materials and Devices*, *2*(4), 470-475.
2. Graewert, M. A., Wilhelmy, C., Bacic, T., Schumacher, J., Blanchet, C., Meier, F., ... & Haas, H. (2023). Quantitative size-resolved characterization of mRNA nanoparticles by in-line coupling of asymmetrical-flow field-flow fractionation with small angle X-ray scattering. *Scientific reports*, *13*(1), 15764.
3. Cinosi, A. , Bolgan, L., & Martelli, C.,(2023). REVIEW ON ANALYTICAL METHODS FOR THE CHARACTERIZATION OF GRAPHENE STRUCTURES AND TOXICITY PROFILES
4. Lee, Y. M., Park, S., & Jeon, K. Y. (2021). Foreign materials in blood samples of recipients of COVID-19 vaccines. *International Journal of Vaccine Theory, Practice, and Research*, *2*(1), 249-265.
5. Vanden-Hehir, S., Tipping, W. J., Lee, M., Brunton, V. G., Williams, A., & Hulme, A. N. (2019). Raman imaging of nanocarriers for drug delivery. *Nanomaterials*, *9*(3), 341.
6. Esmonde-White, K. A., Cuellar, M., & Lewis, I. R. (2022). The role of Raman spectroscopy in biopharmaceuticals from development to manufacturing. *Analytical and Bioanalytical Chemistry*, 1-23. . doi: 10.1007/s00216-021-03727-4
7. Esmonde-White, K. A., Cuellar, M., Uerpmann, C., Lenain, B., & Lewis, I. R. (2017). Raman spectroscopy as a process analytical technology for pharmaceutical manufacturing and bioprocessing. *Analytical and bioanalytical chemistry*, *409*(3), 637-649.
8. Luisetto, M., Edbey, K., Tarro, G., Ahmadabadi, N. B., Cabianca, L., Rasool, M. G., ... & Yurevich, O. (2022). Relevant Article, White Papers and Other Documents Concerning The m RNA VACCINE: An Interesting Collection Useful to Better Understand some Phenomena and to Generate Hypotesys. *International Journal of Forensic Research*, *3*(2), 129-145.
9. Ntziouni, A., Thomson, J., Xiarchos, I., Li, X., Bañares, M. A., Charitidis, C., ... & Diz, E. L. (2022). Review of Existing Standards, Guides, and Practices for Raman Spectroscopy. *Applied Spectroscopy*, *76*(7), 747-772.
10. Buckley, K., & Ryder, A. G. (2017). Applications of Raman spectroscopy in biopharmaceutical manufacturing: a short review. *Applied spectroscopy*, *71*(6), 1085-1116.
11. Wen, Z. Q., Li, G., & Ren, D. (2011). Detection of trace melamine in raw materials used for protein pharmaceutical manufacturing using surface-enhanced Raman spectroscopy (SERS) with gold nanoparticles. *Applied Spectroscopy*, *65*(5), 514-521. DOI: 10.1366/10-06089
12. Rodà, F., Picciolini, S., Mangolini, V., Gualerzi, A., Seneci, P., Renda, A., ... & Bedoni, M. (2023). Raman Spectroscopy characterization of multi-functionalized liposomes as drug-delivery systems for neurological disorders. *Nanomaterials*, *13*(4), 699.
13. Luisetto, M., Ahmadabadi, N. B., Edbey, K. E. K., Cabianca, L., & Llatyshev, O. Y. (2022). Raman Spetroscopy for Biopharmaceutical Quality Control and PAT, Raw Material-Final Products: The Nanolipids Effect on Signal Intensity, Regulatory and Toxicological Aspects. *Jour of Respiratory Med and Clin Pulmonology*, *1*(1), 1-17.
14. Vanden-Hehir, S., Tipping, W. J., Lee, M., Brunton, V. G., Williams, A., & Hulme, A. N. (2019). Raman imaging of nanocarriers for drug delivery. *Nanomaterials*, *9*(3), 341.
15. Luisetto, M., Ahmadabadi, N. B., Ettarhouni, Z. O., Edbay, K., & Latyshe, O. Y. (2022). Monoliths in the mRNA vaccine purification process-The silica resin and other composite

materials: The carbon content. *Virology and Mycology*, *11*(4), 1-14.

16. Luisetto, M., Edbey, K., Tarro, G., & Nili, B. A. (2023). Activated Charcoal and Derivate Materials in Drugs and Biopharmaceutical Purification: Impurity Aspects. *J Mater Sci Nanotechnol*, *11*(1), 105.
17. Cipelli, R. B., Giovannini, F., & Pisano, G. (2022). Dark-field microscopic analysis on the blood of 1,006 symptomatic persons after anti-COVID mRNA injections from Pfizer/BioNtech or Moderna. *International Journal of Vaccine Theory, Practice, and Research*, *2*(2), 385-444. DOI: 10.56098/ijvtpr.v2i2.47
18. Luisetto, Mauro. (2022). book -NEW Biopharmaceutical manifacturing large scale production process- the graphene and derivates role and m RNA vaccine- EXTENDED VERSION.
19. Ki-Yeob J, Sunyoung P, Daniel B, Hyung-Don J. (2023). A Presentation of Analyses of COVID-19 Vaccine Samples, Blood Samples, Urine Samples, Foot Bath Samples, Sitz Bath Samples, and Skin-Extract Samples. 2023 Feb 09; 4(2): 188-217. doi: 10.37871/jbres1663, Article ID: JBRES1663, Available at: https://www.jelsciences.com/articles/jbres1663.pdf
20. Luisetto, M., Edbey, K., Tarro, G., Ahmadabadi, N. B., Cabianca, L., Rasool, M. G., ... & Yurevich, O. (2022). Relevant Article, White Papers and Other Documents Concerning The m RNA VACCINE: An Interesting Collection Useful to Better Understand some Phenomena and to Generate Hypotesys. *International Journal of Forensic Research*, *3*(2), 129-145.