Mini Review
Volume 11:9, 2022

Pharmaceutical Regulatory Affairs: Open Access

ISSN: 2167-7689 Open Access

Raman Spetroscopy for Biopharmaceutical Quality Control and PAT, Raw Material-Final Products: The Nanolipids Effect on Signal Intensity, Regulatory and Toxicological Aspects

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Abstract

Biopharmaceuticals production is based on a GMP system of quality control used for the regulatory scope. Relvant for this role the analytical procedure, specificity and sensibility of the methods to test raw materials but also the final products before commercialization. Aim of this work is to verify the role played by nanolipids on Raman Spettroscopy encapsulating active principle or other substantia unsing different procedure: 1) Destructive and 2) Non destructive technique. This is relevant because regualtory agency authorized (EMA) for cGMP rules the use also of non destructive methods like RAMAN spettroscopy in various stage of manifacturing drugs (for raw material and final product).

Keywords: Biopharmaceuticals • m RNA vaccine • GMP • European pharmacopeia • EMA procedures • PAT • Quality control • Raw material • Final products • Destructive • Non destructive direct methods • Intensity of signal • Sample pre-treatement of the sample • Extraction • Toxicology

Introduction

In last decades raman spetroscopy was deeply introduced in various settings and also in pharmaceutical

Drugs production because innovative, non invasive and easy to use technology (Figure 1).

This work start with the interesting facts that some single researcher finded graphene deivates in some vials of m RNA COVID-19 vaccine but this whas not confirmed by regulatory agency that written:

In as official document (EMA): Last updated: 27 January 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:

"In the EU a marketing authorisation is granted to a medicinal- product only after its quality, safety and efficacy have been evaluated and a positive benefit-risk balance related to its use has been concluded. For EU authorisations of COVID-19 vaccines this assessment is carried out by the European Medicines Agency.

EMA has analysed reports describing the analysis of several vials of COVID-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 from the European Directorate for Quality of Medicines and the independent national testing laboratories responsible for the batch release (OMCLs).

Graphene oxide GO is not used in the manufacture or formulation of any of the COVID-19 vaccines or other medicines, so it would not be present at manufacturing -facilities and there is no obvious way that it could get into the vaccines. 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 to the release. No product complaints have been received for the batches mentioned in the paper. The presence of graphene or graphene derivatives in the vaccines therefore are not plausible.

The Commission and EMA do not consider that any further- actions are necessary at this stage."

But if we read the work of one of this researcher: Campra P [1]

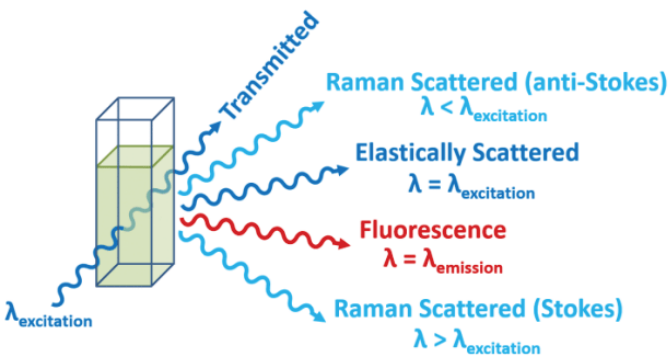

Figure 1. Fluorescence, elastic scattering and Raman scattering processes.

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Date of Submission: 18 August 2022; Manuscript No. pbt-22-72271, **Editor assigned:** 20 August 2022, PreQC No. P-72271; **Reviewed:** 03 September 2022, QC No. Q-72271, **Revised:** 09 September 2022; Manuscript No. R-72271; **Published:** 17 September 2022, DOI: 10.37421/2167-7689.2022.11.328

Analytical methodology

"Fundamentals of the micro -Raman technique Due to the characteristics of the sample and to the dispersion of objects with a graphene appearance of micro-metric size in a complex matrix of indeterminate composition, the direct application of spectroscopic methods does not allow characterization of the nano-particles studied here without a previous microscopic- localization or fractionation from the original sample.

Therefore, microscopy coupled to raman spectroscopy (micro-RAMAN) was selected as an effective technique for an exhaustive screening of micrometric objects visible under the optical microscope." [1]

So it is possible to verify that in example Young RO [2] reported a pretreatment of the sample before test with

Other technique

Young RO [2] Scanning & Transmission Electron Microscopy Reveals Graphene Oxide in CoV-19 Vaccines.

"Steps of Analysis of Vaccine Aqueous Fractions

Refrigerated samples were processed under sterile conditions, using laminar flow chamber and sterilized lab ware.

Steps for analyses were:

1. Dilution in 0.9% sterile physiological saline (0.45 ml + 1.2 ml)

2. Polarity fractionation: 1.2 ml hexane + 120 ul of RD1 sample

3. Extraction of hydrophilic- aqueous pHase

4. UV absorbance and fluorescence spectroscopy scanning" [3]

It can be considered a distructive method.

And according the Eurpean pharmacopeia: Among the methods established for quality control of classical medicines the so called "non-invasive", e.g., non-destructive, techniques, such as near-infrared and Raman spectroscopy have been applied for molecular imaging and analytics in process analytical technology (PAT) and are implemented in quality by design (QbD) concepts

But What is Raman Spectroscopy?

Raman spectroscopy is an chemico - analytical technique where scattered light is used to measure the vibrational energy modes of a sample. It is named after the Indian physicist researcher C.V. Raman who, together with his research partner K. S. Krishnan, was the first to observe Raman scattering in the 1928.

Raman spectroscopy can provide both chemical- structural information, as well as the identification of substances through their characteristic Raman 'finger-print'. Raman spectro-scopy extracts this information through the detection of Raman scattering from the sample.

This method is based on the phenomena of diffusion of an electro-magnetic mono- cromatic radiation laser

By the sample tested.

It is obvious that it is relevant to separate the sample from its chemical contest in order to avoid other shade or interference form other molecule inside the same sample.

This last molecule make possible not to obtain reviability results.

For this reason often it is used to extract the analite to be detected with solvent – or diluent before test.

And What is the Raman Scattering?

When light is scattered by molecule, the oscillating electro magnetic field of a photon induces a polarisation of the molecular -electron cloud which leaves the molecule in a higher energy- state with the energy of the photon transferred to the molecule. This can be considered as the formation of a very short-lived complex between the photon and molecule which is commonly named the virtual -state of the molecule. The virtual state is not stable and the photon is re-emitted almost immediately, as scattered light.

In the vast majority of scattering events, the energy of the molecule is un-changed after its interaction with the photon; and the energy, the wavelength, of the scattered photon is equal to that of the incident photon. This is named elastic (energy of scattering particle is conserved) or Rayleigh scattering and is the dominant process.

In a much rarer event (1 in 10 million photons)Raman scattering occurs, which is an inelastic scattering process with a transfer of energy between the molecule and scattered photon.

If the molecule gains energy from the photon during the scattering (excited to a higher vibrational- level) then the scattered photon loses energy and its wave-length increases which is called Stokes Raman scattering. Inversely, if the molecule loses energy by relaxing to a lower vibrational level the scattered photon gains the corresponding energy and its wave length decreases; which is called Anti-Stokes Raman scattering. Quantum mechanically Stokes and Anti-Stokes are equally likely processes. With an ensemble of molecules, the majority of molecules will be in the ground vibrational level (Boltzmann distribution) and Stokes scatter is the statistically more probable process. As a result, the Stokes Raman scatter is always more intense than the anti-Stokes and for this reason, it is nearly always the Stokes Raman scatter that is measured in the Raman spectroscopy (Figure 2).

Raman shift

It is clear from the above figure, that the wavelength of the Raman scattered light will depend on the wavelength of the excitation light. This makes the Raman scatter wavelength an impractical number for comparison between spectra measured using different lasers. The Raman- scatter position is therefore converted to a Raman shift away from excitation wave length:

Raman shift equation: The first term is the wave number Raman shift in cm-1, $\lambda(0)$ is the wave length of the excitation laser in nm, and $\lambda(1)$ is the wave length of the Raman scatter in nm.

Vibrational Modes

Figure reported shows that Raman- spectroscopy measures the energy gap between the vibrational levels of the molecule. The ladder of vibrational-levels shown in Figure 2 is for a single vibrational mode of the molecule. Poly-

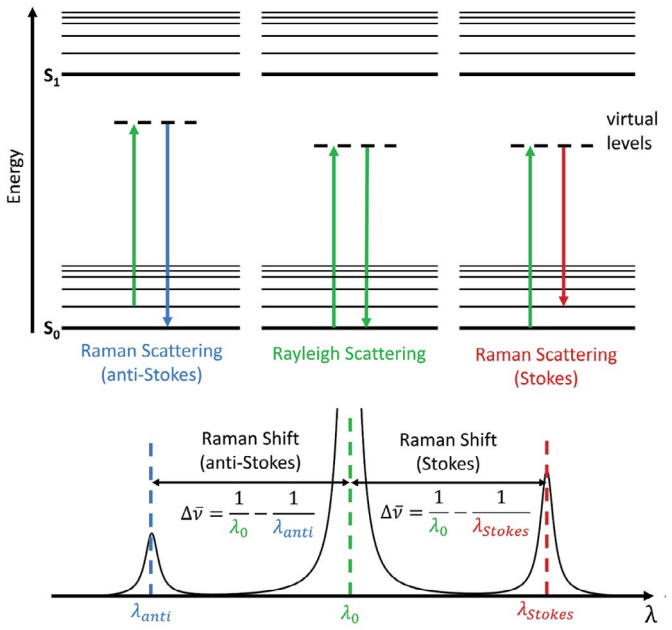


Figure 2. Jablonski Diagram showing the origin of Rayleigh, Stokes and Anti-Stokes Raman Scatter.

atomic molecules will contain many vibrational modes, each with their own ladder of vibrational- levels (Figures 3 and 4).

Applications

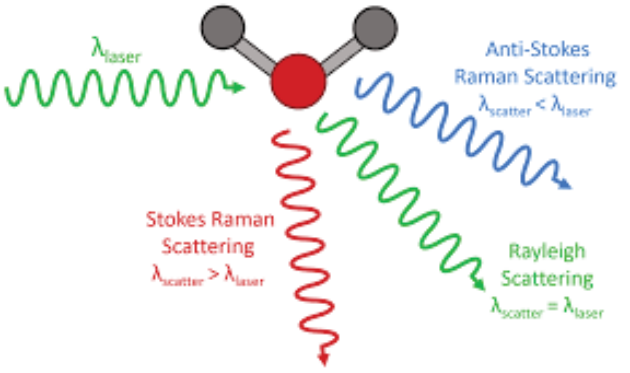
Raman spectro-scopy is commonly used for qualitative and quantitative applications and can be applied to solid, liquid and gaseous samples. Raman spectro-scopy is a rapid and non-invasive analytical method and can be performed off-line, at-line, on-line or in-line, e.g. for process analytical technology (PAT)- Process analytical technology. Raman spectrometers can be situated far from the point of measurement using long-distance optical fibres to collect the Raman signal. Raman spectro-scopy has a wide variety of applications, for example:

- Identification of materials, active substances or excipients;
- Determination of solid-state properties, polymorphism and solvated state;
- Quality control, assay, uniformity of dosage units;
- Process analysis, monitoring of biological and chemical reactions, synthesis, crystallisation, granulation, mixing, drying, lyophilisation, extrusion, encapsulation and coating;
- Detection of falsified products;
- Mapping, imaging and depth profiling of pharmaceutical forms, distribution of chemical compounds, detection of un-known substances.

Equipment

2 types of Raman spectrometers can be distinguished depending on the detection principle, dispersive and Fourier transform (FT) instruments. These may be benchtop instruments (including microscope-coupled devices, portable -instruments) or hand-held instruments.

Response-intensity scale: The absolute and relative intensities of



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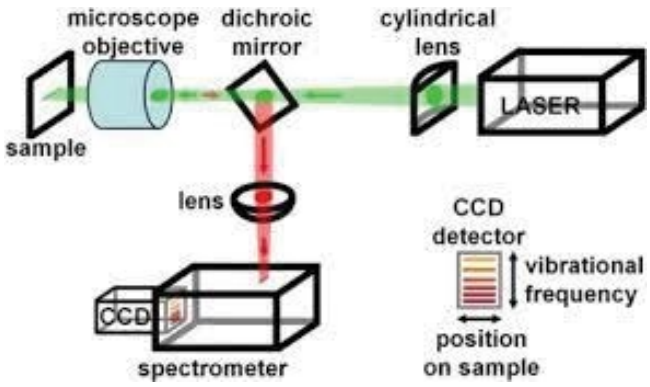


Figure 3. Raman spectroscopy and related techniques in biomedicine by Andrew Downes (Alistair Elfick School of Engineering, The University of Edinburgh, Edinburgh EH9 3JL, UK).

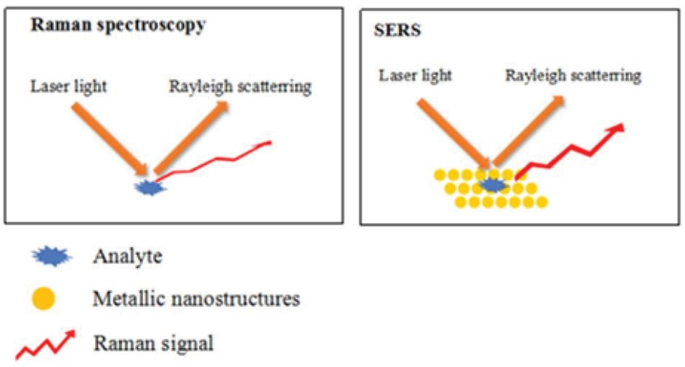


Figure 4. Food microbiology detection of foodborne pathogens by surface enhanced raman spectroscopy.

Raman signals are affected by variations of several factors including:

- Polarisation of the irradiating -light
- Polarisation of the Raman scattered -light
- Intensity of the irradiating- light
- Instrument response
- Focus and geometry at sample
- Packing density of particles in solid samples
- Refractive index n or change of n (△n) between analyte and the environment
- The particle- size and particle-size distribution
- The scattering cross-section
- The absorption cross-section

The verification of the response-intensity scale is principally performed for quantitative- methods.

Procedure

Preparation of the sample: Raman spectra can be obtained from solids, liquids or gases directly, in suitable glass or plastic containers or through films (provided that un-wanted signal contributions are under control), generally with-out prior the sample preparation or dilution.

Qualitative methods: Since frequency shift positions are employed for identification, identical laser intensity for both the reference standard and the material to be examined may not be necessary. The material to be examined is measured in the same physical -state (liquid, solid) as the reference or library material. Raman techniques offer the advantage of non-invasive measurements of the material to be examined with-out removal from the packaging. Some packaging materials may lead to additional signals in the Raman- spectrum. This is especially the case when the packaging material absorbs at the laser's excitation wave-length.

Quantitative methods: Quantitative determination requires that the reference – standard RS and the material to be examined must be measured at the same laser -intensity and frequency. Ensure that the material to be examined is measured in the same physical state (liquid, solid) and concentration range as the reference standard or library used for calibration. While the Beer-Lambert law is not valid for Raman spectroscopy, Raman -intensity is directly proportional to the concentration of the Raman scattering analytes; For solid samples and suspensions the Raman intensity may be affected by the matrix (owing to fluorescence and self-absorption). The Raman signal is influenced by the refractive- index of the material, the particle size and the particle-size distribution (where small -particles give a relatively more-intense Raman scattering than the large particles), the packing density, the scattering cross-section, the absorption cross-section."

Material and Methods

With an observational method some relevant scientific literature and (Figures 1-16) are reported and then analizyed.

After this review and experimental project hypotesys is submitted in order to provide a complexive glogal conclusion related the topic of this article.

All literature comes from scientific bio medical database.

Results

"Adoption of Quality by Design (QbD) principles, regulatory support of QbD, process analytical technology (named PAT), and continuous manufacturing are major factors effecting new approaches to pharmaceutical manufacturing and bio processing. In this review work, we highlight new technology developments, data analysis models, and applications of Raman spectro-scopy, which have expanded the scope of Raman spectro-scopy as a process analytical technology. Emerging technologies such as transmission and enhanced reflection Raman, and new- approaches to using available technologies, expand the scope of Raman spectroscopy in pharmaceutical manufacturing, and now Raman spectro scopy is successfully integrated into real-time release testing, continuous manufacturing, and statistical process control. Since the last major review of Raman as a pharmaceutical PAT in 2010, many new Raman applications in bio processing have emerged. Exciting reports of in situ Raman spectroscopy in bi-oprocesses complement a growing scientific field of biological and bio-medical Raman spectroscopy. Raman spectroscopy has made a positive impact as a process analytical and control tool for pharmaceutical manufacturing and bio processing, with demonstrated scientific and financial benefits throughout a product's lifecycle. Raman spectroscopy is an optical spectroscopy technique that provides a "molecular finger-print" of a sample.

As optical- method, Raman enables non-destructive analysis of chemical composition and molecular structure. Applications of Raman spectro scopy in polymer, pharmaceutical, bio processing, and biomedical analysis have surged in the past three decades as laser sampling and detector technology has improved. Because of these technological advances, Raman spectro-scopy is a practical analysis technique inside and outside the laboratory. Raman spectro scopy is an established PAT tool. Since 1980s, Raman spectroscopy has been used to study active pharmaceutical ingredients (API). Raman spectroscopy as a tool for API analysis has been described for many applications, as polymorph identification, quantitative analysis, in situ crystallization monitoring, real-time release testing, pharmaceutical unit operations, and process-induced transformations. In addition to identifying isolated polymorphic forms, mixtures of forms can be analyzed and quantified. The diverse structures that have been measured by Raman, from the discovery laboratory to the manufacturing environment, show that Raman can reliably provide quantitative data. In-line

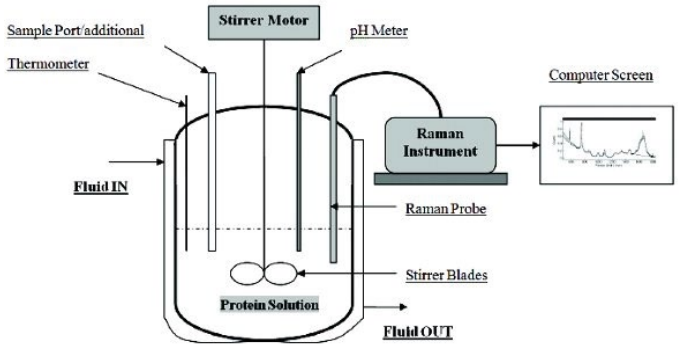


Figure 5. Use of in-line Raman Spectroscopy as a non-destructive and rapid analytical technique to monitor aggregation of a therapeutic protein.

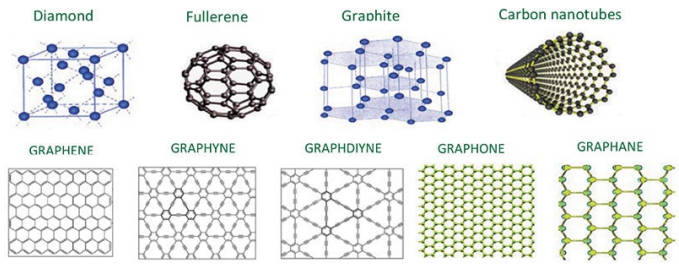
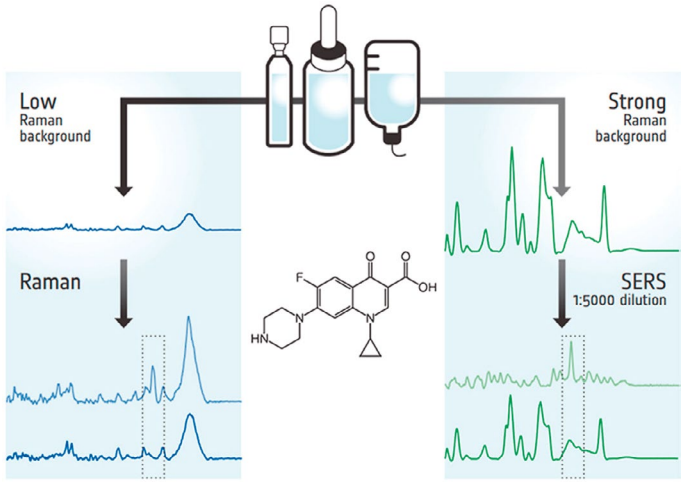


Figure 6. Raman Spectroscopy: a non-destructive, non-contact and simple technique to characterize carbon materials-part 1: Carbon nanotubes.



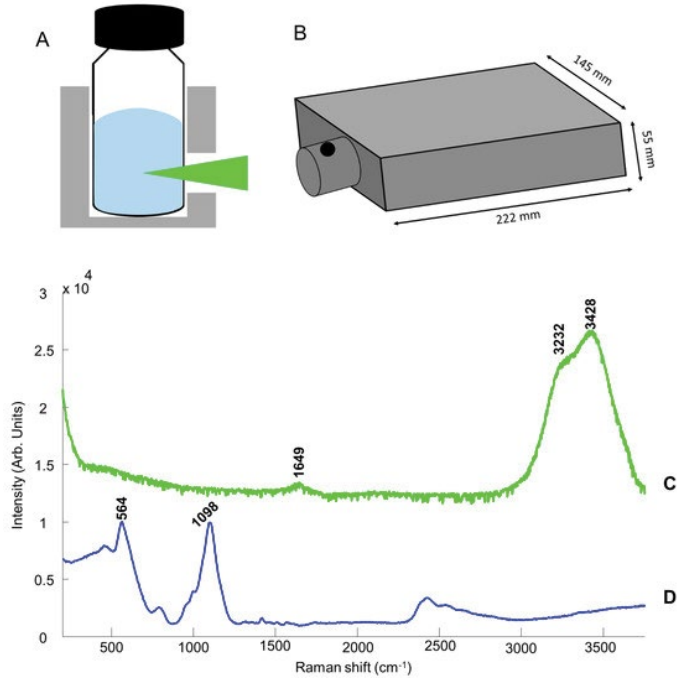


Figure 7. Illustration of the portable Raman device used: (A) sample holder, (B) the device, (C) mean Raman spectrum of the deionised water collected from a glass vial, and (D) a Raman spectrum of glass.

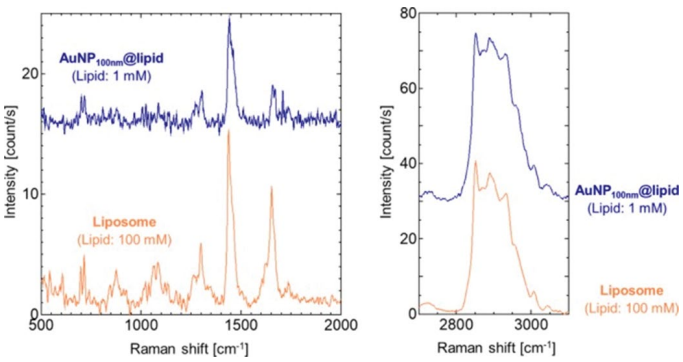


Figure 8. Raman spectra of AuNP100nm@lipid (blue) and liposome (orange), obtained wit h total lipid concentrations of 1 and 100 mM, respectively. Lipid- compositions were DOPC/Chol (60/40). All the samples were measured at 25°C. At least three reproducible spectra were obtained for each system. Raw spectral data are shown in the supporting information .

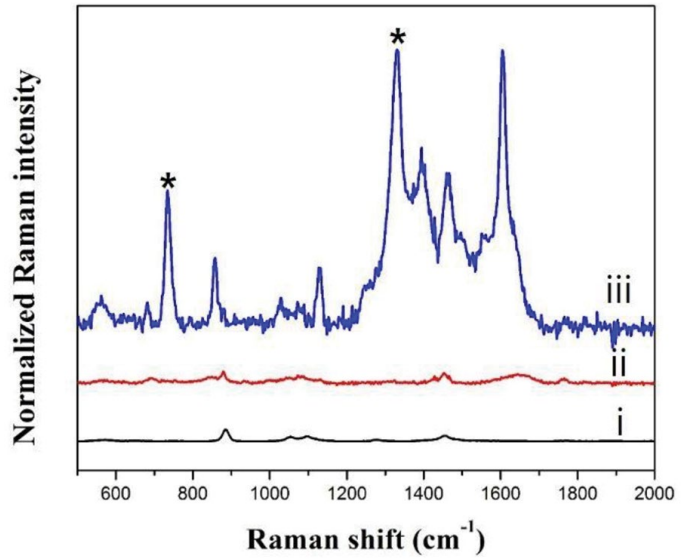


Figure 9. Surface enhanced Raman scattering (SERS) spectra of (i) SiO_2@Au@Ag NPs, (ii) 10 mM ATP, and (iii) SiO_2@Au@Ag NPs in the presence of 10 mM ATP. The concentrations of SiO_2@Au@Ag NPs were 1 mg/mL in ethanol solution, respectively.

Raman spectro scopy can control critical process parameters, enables real-time process corrections, and ensures consistent production of the correct API form. We highlight the new applications in API synthesis and crystallization, real-time release testing, flow or continuous manufacturing, and new developments in Raman spectroscopy for understanding and controlling bio processes Regulatory perspectives and also guidance.

A philosophical shift in pharmaceutical manufacturing quality, which is strongly encouraged by regulatory agencies, has created opportunities to integrate real-time process analytics into manufacturing processes. In 2002, the U.S. FDA launched an initiative to encourage innovation in manufacturing technology and quality system approaches. The FDA 2004 PAT framework strongly emphasized a shift from tested-in quality after the drug product was produced to building in quality throughout production with "continuous real time quality assurance". The European Medicines Agency established a PAT team in 2003, which released guidance documents on process PAT, quality by design (QbD), and real-time release testing. International Conference on Harmonization (ICH) Q8, Q9, Q10, and Q11 documents reinforced FDA and EMA guidance, which has been implemented in the USA, European Union EU, and Japan since 2009.

The FDA and ICH documents provided a strategic- guidance, rather than prescriptive guidance, on developing an approach to understand and manage the risks that might affect critical quality attributes. PAT has an important role in this new framework to understand and manage risk throughout a pharmaceutical product's lifecycle. Recently, these principles were extended to bio processing. As a PAT in pharmaceutical manufacturing and bio processing, Raman spectro scopy has demonstrated value from scientific understanding to process control. Over the past 25 years, Raman spectroscopy instrumentation has evolved from home-built academic lab. instruments to robust commercially available solutions-based systems. The advent of stable laser sources, high-speed optical fibers, volume holographic gratings, and low-noise charge coupled device detectors enabled robust commercial Raman- spectroscopy instruments. Newer commercial instruments are straight-forward to use because they do not require constant realignment or sophisticated knowledge of optics, are equipped with instrument control software, and are integrated with Raman spectral libraries. Thus, Raman spectroscopy is accessible to scientists and environments beyond the academic research environment world. Modern instrumentation has been reviewed in detail elsewhere. Briefly, there are three basic components of a Raman spectro-graph, including a laser, sampling optics, and detector. Modern Raman instrumentation optimizes the amount of inelastically scattered -photons and their detection. Modern Raman instruments use a laser as the illumination source because it is a high-intensity mono-chromatic source of light. While the laser wavelength can vary from the UV to the near-infrared (λ = 200–1064 nm), most pharmaceutical or bio-processing applications use near-infrared wavelengths (λ = 785 or 830 nm), primarily to minimize fluorescence interferences.

Articles, bubbles, or droplets with sizes approaching the excitation wavelength exhibit Lorenz-Mie scattering, which causes aqueous systems to become turbid. Photons can be scattered multiple times, resulting in photons being diffusely distributed in a turbid media. API or excipient particles and cellular organelles, like mitochondria and nuclei, also strongly scatte-r light. Understanding photon -transport in turbid media is an important consideration for quantitative Raman spectroscopy applications in content uniformity, real-

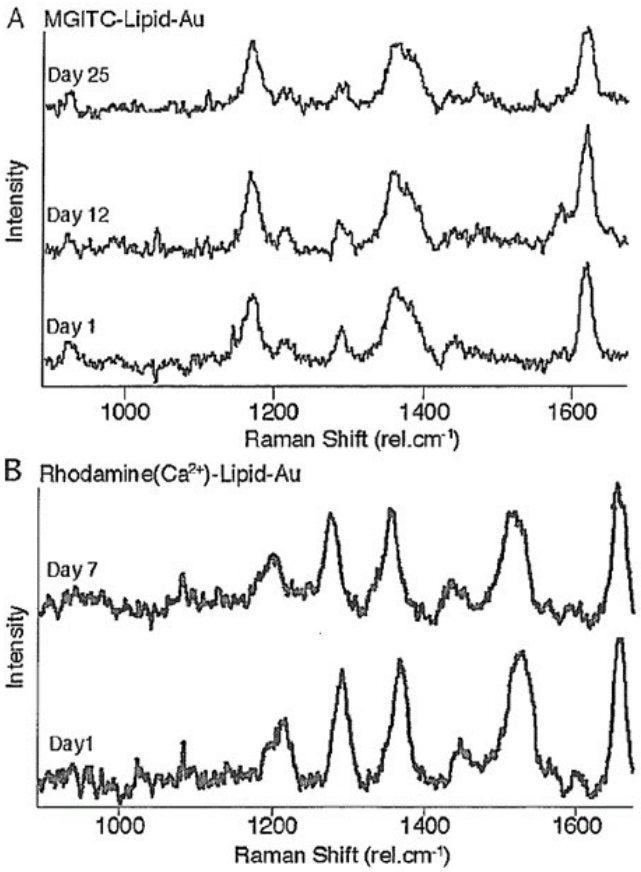


Figure 10. Shows the stability of MGITC-lipid-coated particles and Rho-lipid-coated-particles in which: A) shows the SERS spectrum of MGITC-lipid-coated particles collected on day of synthesis, 12 days, and since 25 days after synthesis; and B) shows the SERS spectrum of Rho-lipid-coated-particles collected on day of synthesis, and 7 days after synthesis, in which for both cases, (particles were stored in water at 4 deg C. between measurements)

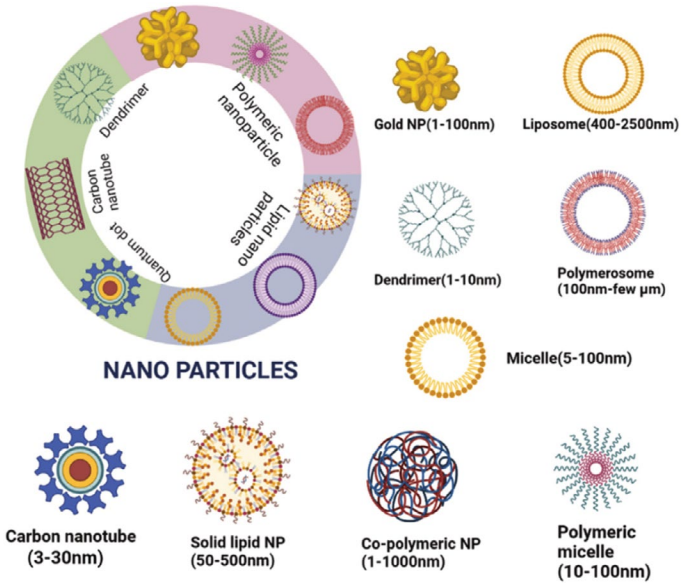


Figure 11. Raman imaging of nanocarriers for drug delivery.

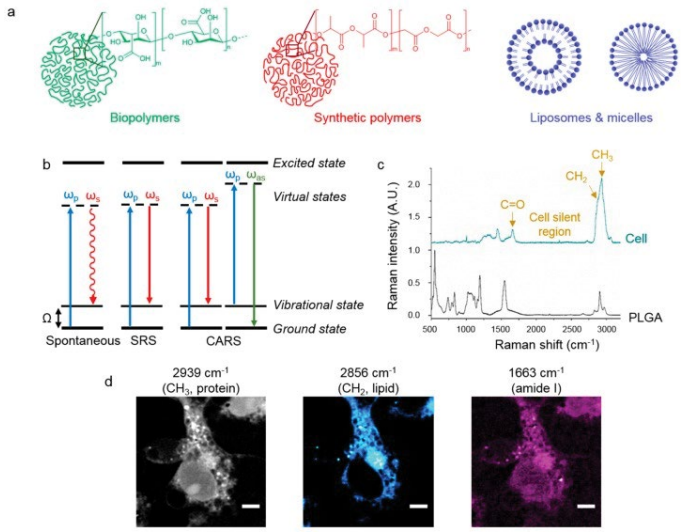


Figure 12. Raman imaging of nanocarriers. (a) Representation of different materials which can be fabricated into nanocarriers, such as biopolymers (alginate), synthetic polymers (PLGA), and lipids (as liposomes and micelles). (b) Energy level diagrams showing the processes of spontaneous Raman, stimulated-Raman scattering (SRS), 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). The Spectra are normalized and offset for clarity. (d) SRS images of microglia when Ω=2939 cm^{-1} (CH_3, proteins, grey), 2856 cm^{-1} (CH_2, lipids, cyan), and 1663 cm^{-1} (amide I, magenta). Scale bars=5 µm.

time release testing, and in situ bio process control. Much research has been devoted in developing Raman spectroscopy for pharmaceutical solids analysis, taking into consideration process compatibility, validation, and ease of use. Figure 5 reported shows the variants of Raman spectro scopy that utilize fiber optic probes. Within the process environment, the sampling flexibility of Raman spectroscopy means that Raman can be employed as an off-line, at-line, on-line, or in-line (or in situ) PAT. Pharmaceutical excipient chemical and physical -properties are typically a critical process parameter because they affect manufacturability, bio-availability, and risk of process-induced API transformations. Raman spectroscopy measures excipient material attributes non-destructively and rapidly, with handheld systems typically used for this application. A comprehensive database of commonly used pharmaceutical excipients contains both the Raman spectrum and band assignments. The excipient spectrum can be affected by different crystal forms, amorphous -content, or process variations. In-house preparation of excipients or bio pharmaceutical formulations may require its own risk-based manufacturing approach." [4]

"A 785 nm diode -laser and probe with a 6 mm spot size were used to obtain spectra of stationary powders and powders mixing at 50 rpm in a high shear convective blender. 2 methods of assessing the effect of particle characteristics on the Raman sampling depth for micro-crystalline cellulose (Avicel), aspirin or sodium nitrate were compared: (A) the information depth, based on the diminishing Raman signal of TiO_2 in a reference plate as the depth of powder prior to the plate was increased, and (B) the depth at which a sample became infinitely thick, based on the depth of powder at which the Raman- signal of the compound became constant. The particle size, the shape, density and/or light absorption capability of the compounds were shown to affect the "information" and "infinitely thick" depths of individual compounds. When different sized -fractions of aspirin were added to Avicel as the main component, the depth values of aspirin were the same and matched that of the Avicel: 1.7 mm for the "information" depth and 3.5 mm for the "infinitely thick" depth. This latter value was considered to be the minimum Raman sampling depth when monitoring the addition of aspirin to Avicel in the blender. Mixing profiles for aspirin were obtained non-invasively through the glass- wall of the vessel and could be used to assess how the aspirin blended into the main component, identify the end point of the mixing process (which varied with the particle size of the aspirin), and determine the concentration of aspirin in real time. The Raman procedure was compared to 2 other non-invasive

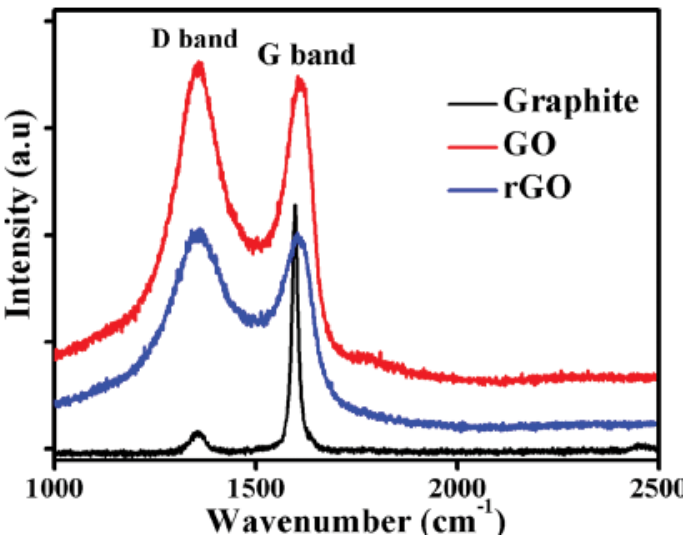


Figure 13. Measured Raman spectra of graphite, graphene oxide, and reduced graphene oxide.

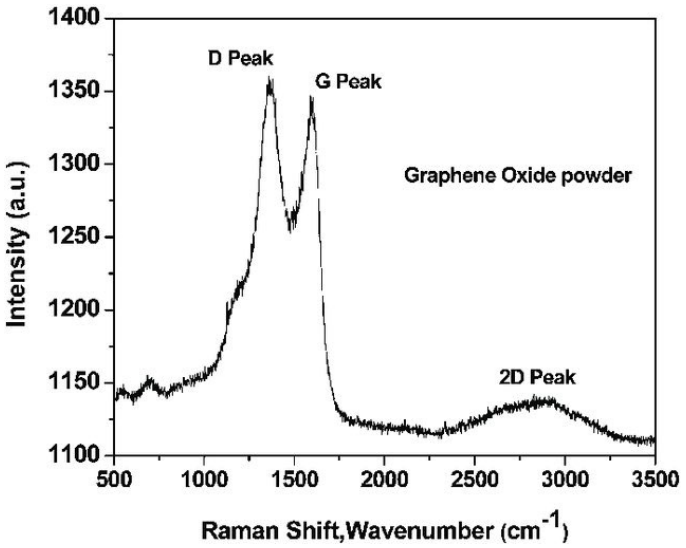


Figure 14. Measured Raman spectra of graphene oxide powder.

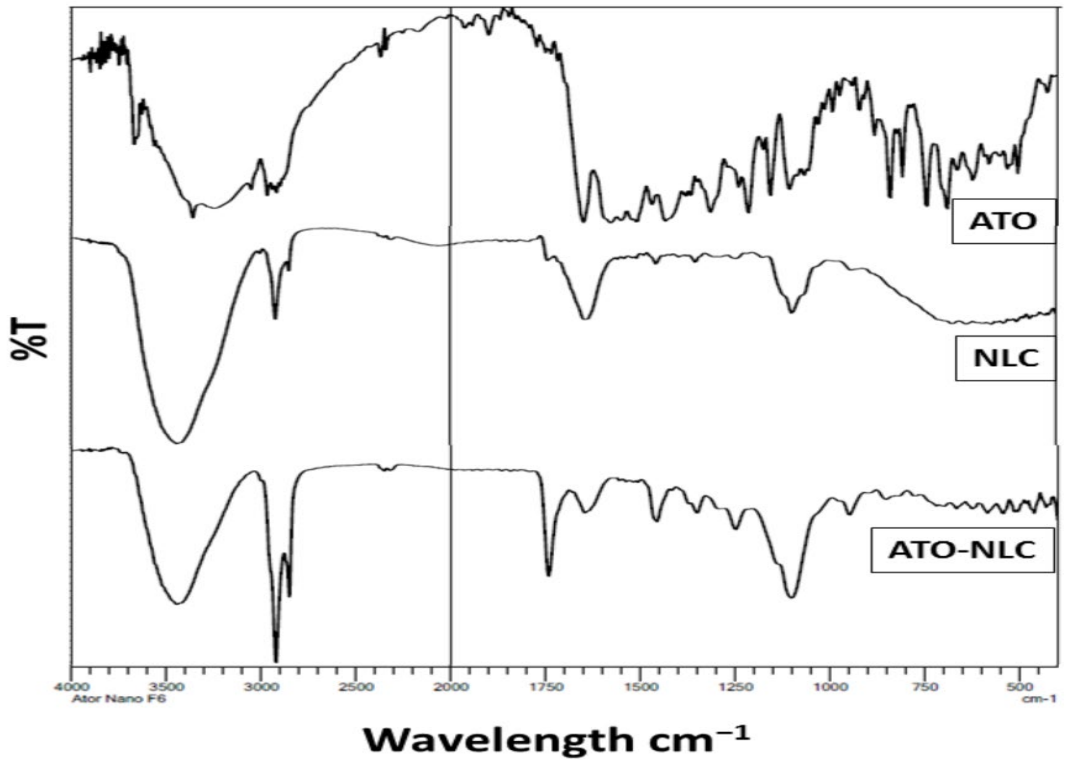


Figure 15. FTIR spectra of pure ATO (atorvastatin), free NLC (Nano structured lipid carrie)and optimized ATO-NLC formulation.

monitoring techniques, near infrared (NIR) spectro-metry and broadband acoustic emission spectro-metry. The features of the mixing profiles generated by the three techniques were similar for addition of aspirin to Avicel. Even if Raman was less sensitive than NIR spectrometry, Raman allowed compound specific mixing profiles to be generated by studying the mixing behaviour of an aspirin–a Highlights

Powder blending monitored non-invasively by wide area Raman spectro metry. Effect of particle size on sampling depth and Raman signal investigated for wide area illumination. Raman measurements used to monitor mixing dynamics, determine end-point and perform quantitative analysis. Higher chemical specificity of Raman compared to near infrared- spectrometry offers advantages for multi-component mixtures spartame/Avicel mixture [5] (Figure 5 and 6)."

Why Raman spectroscopy has been used?

Advantages of Raman spectroscopy:

- Very small samples
- No special preparation of samples

Ease of use:

- Non-destructive and non-contact analysis
- Measurement of various types of samples (liquids, solids, powders, etc.)
- Raman Spectro scopy needs relative short time. So we can do Raman Spectro scopy detection very quickly.
- Raman spectro scopy is one of the most informative probes for studies of material properties under extreme conditions of high pressure and low- temperature
- Depth analysis:

    1. Raman Spectroscopy for Pharmaceutical Analysis & Quality Control
    2. Raman spectroscopy helps ensure quality along the pharma supply chain of materials—from incoming raw materials through to finished product.

Jacques Ledru, Head of Characterization, Catalent, Nottingham 2021

"Raman spectroscopy has many applications within the pharmaceutical industry. It can be used to identify polymorphs,in example, and to analyze active pharmaceutical ingredient (API) forms and their distribution within formulated -products. But what is it, and how can it be applied in practice?

In contrast to standard infrared (IR)- spectro-scopy, which identifies the specific frequencies of radiation that are absorbed by a sample, Raman spectro-scopy studies the way light is scattered by the molecules. As a laser beam passes through the sample, much of the light passes through and scatters with its energy unchanged; this is known as Rayleigh- scattering.

Some of its photons collide with the molecules and lose energy, in a phenomenon known as a Stokes -shift. Others may pick up energy from excited molecules and emerge with a higher energy level, or an anti-Stokes shift. In Raman spectro-scopy, the light that emerges is collected, and that which is scattered without changing energy is filtered out. What remains provides a unique spectral pattern for that individual molecule. This finger print can be used to identify the molecule by comparing the pattern to an knowed reference.

Transmission Raman spectrometry, mean while, often gives better results when sampling solids than a conventional backscatter Raman technique as the radiation passes through the sample analyzing a much larger volume. As the technique is a non-evasive and non-destructive, it can be used for the direct analysis of batches of hundreds of whole tablets or capsules that can be scanned in minutes, and can quantify both the API (down to less than 1% drug loading) and the excipient in a single measurement using appropriately developed partial least-squares calibration- models.

In this technique, the incident light is passed through an objective - lens, and focused onto a very small spot. This allows resolution down to fractions of a micron to be achieved. The distribution of components within a sample can be determined in this way, the laser can be focus on the sed on specific areas of concern. This may be to determine the presence/identification of a suspected contaminant, particle or other un expected feature, and as such, Raman microscopy is much more sensitive than techniques used for the analysis of a material's bulk properties."

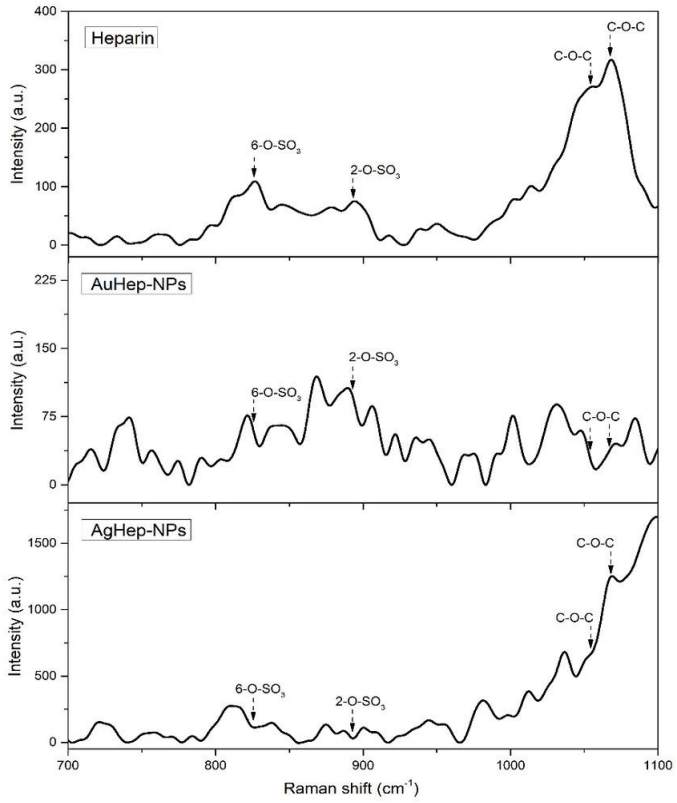


Figure 16. Raman spectra of heparin and those of AuHep-NPs and AgHep-NPs.

Raman-based detection of ciprofloxacin and its degradation in pharmaceutical formulations

Chen Liu Lisa Müller-Böttticher ChangLiude Jürgen Poppa Dagmar Fischerg Dana Cialla-Mayab

"A Raman-based label-free analytical method was developed to detect the antibiotic ciprofloxacin (CIP) in various pharmaceutical formulations in the presence of different matrices (ear drops, eye drops and infusion- solutions)" (Figure 7).

"European Pharmacopoieia (Ph. E.), provides the legislative framework for product testing and regulatory- bodies such as the European Directorate for Quality of Medicines (EDQM) prequalify methods for these purposes, including the biological- standards to be used to obtain comparability. Between the methods established for quality control of classical medicines the so called "non-invasive", e.g., non-destructive, techniques, such as near-infrared and Raman- spectroscopy have been applied for molecular- imaging and analytics in process analytical technology and are implemented in quality by design (QbD) concepts.

Recent technical developments in the field of the Raman - technology now enable manufacturers to use this technique for analysis of more- complex biological products including protein mixtures in bio reactors and cell-based and tissue-engineered products. Raman -micro spectroscopy is an inelastic light scattering-based method useful for the non-destructive analysis of biochemical samples. It provides a wealth of molecular information on a specimen by the sample's own inherent vibrational -signatures.

As the bio-chemical composition of a sample is mirrored in the Raman spectrum, mathematical methods including analytical modelling translate the physically recorded Raman data into higher level information, which can further be exploited for comparative analyses. The fingerprint-like specificity of spectral -signatures can be utilized to setup a reference database of tested biological -products for identification purposes" [6,7].

Raman spectroscopy as a process analytical technology (PAT) in bioprocessing

"Advances in cell -engineering, process control, and media composition are credited with improving the volumetric yield of cell- culture bio processes, making bio pharmaceutical manufacturing more cost-effective and practical. Adoption of PAT and Quality by Design (QbD) principles is an important contributor to improvements in bio- process control. PAT provides real-time understanding which helps to manage risk throughout a bio pharmaceutical product's lifecycle. The PAT- framework is an integrated approach using historical process knowledge, modeling, and analyses. Many types of physical and chemical analyses are used for bio processing. Traditional parameters such as pH, temperature, dissolved oxygen, feed composition, and feed timing are measured in situ. Bio-chemical -parameters such as nutrients, metabolites, amino acids, proteins, cell viability, and biomass can be measured by spectroscopy, electro-chemical sensors, bio-chemical assay, or chromatography. These biochemical PATs can be used in situ, integrated with an automated sampler for at-line measurements, or off-line. Spectroscopy- PAT techniques are based on light's interactions with materials. They provide a fast, label-free, non-invasive, and non-destructive chemical analysis of a material" (Figure 8).

"Preparation of ATP-Encapsulated Liposomes and SiO_2@Au@Ag NPs

We designed and fabricated ATP-en-capsulated liposomes that could release ATP only when the liposome structure was ruptured for SERS-based immuno-assays as shown in the Scheme reported. For this, ATP en-capsulated lipo-somes and gold-silver alloy (Au@Ag)-assembled silica NPs (SiO_2@Au@Ag) were prepared, separately. Both the liposomes and SiO_2@Au@Ag NPs alone were inactive for SERS -measurement. When the liposome's structure is broken, and the ATP is released, a strong SERS signal could be obtained, because the released ATPs are immobilized on SiO_2@Au@Ag NPs." (Figures 9-12).

"There are various ways of fabricating materials into nano carriers, depending on the desired properties of the final formulation and the drug to be en-capsulated. Often, the polymer is dissolved in an organic solvent prior to emulsification with an aqueous phase to form nano-sized droplets, which become the nano carriers upon evaporation of the organic solvent. Hydrophobic drugs can be added into the organic phase with the polymer, whilst the process can be modified to a double water-in-oil-in-water emulsion to encapsulate hydrophilic drugs. Liposomes are generally formed by a lipid -film hydration method, and micelles will self-assemble in an aqueous -solution above the critical micelle concentration " (Figures 13-16).

Experimental project hypotesys

In order to verify the absence/presence of graphe ederivates in vials of some bio- pharmaceutical compounds it is needed to test 100 sample of a new technological products (In example m RNA vaccine in nanolipids).

This using analitycal procedure officially CGMP approved (RAMAN spetroscopy) and with the accettable sensibility. (one procedure with a classic destructive method and using also a non destructive method).

1) Method as approved Europena Pharacopoeia like direct non desctructive method

2) Method as reported by some rearcher (with extraction in a classic chemical methods befor test, destructive method)

This sample must divided in group of 20 and sended blinded to various and different accreditated chemical laboratory and independent.

It is needed a control group, all sample blinded.

The sample must to be tretated for the pre-analitycal need (extraction) before to be analyzed.

This in order to verify in the same condition the inside nanolipids included and outiside of this.

Results

Verify is there is or not significative presence of graphene or its derivated in the final approved vials. ($p < 0,005$). The results must to be divided using a destructive method and a non destructive one.

Discussion

It is interesting to observe the analitical behavior of nanoparticles-liposome with encapsulated molecule in a RAMAN spectra related the non encapsulated ones.

Observing Figure 8 it is possible to say that encapsulated particle produce a reduced intensity in Raman Spettroscopy.

The heparin molecule show greater intensity signal vs the heparin AU -hep - NPS

Also of interst to observe the kinetics during time of some nanoparticles as reported and the fact that

After 1 -12 -25 days the signal gradually increase.

Of great interest the fact that some researcher (as published by Young R.O) using other method

Pre- treated the sample in order to have extraction before test.

P. Campra associate professor ALMEIRA university Phd in Chemical sciences written:

"Fundamentals of the micro -Raman technique Due to the characteristics of the sample and to the dispersion of objects with a graphene appearance of micro-metric size in a complex matrix of indeterminate composition, the direct application of spectroscopic methods does not allow characterization of the nano-particles studied here without a previous microscopic- localization or fractionation from the original sample."

According Vanden-Hehir S, et al. [8] "A major advantage of Raman is that it allows direct imaging of the nanocarriers, and not the payload en-capsulated within them."

EMA procedure (GMP) for quality control of final drugs and raw material write on its EUROPEN PHARMACOPEIA report that it can be used for classical drugs CQ -RAMAN SPETTROSCOPY aslo in non destructive direct method.

But because as reported in the "Assesment report "of a famous m RNA COVID-19 VACCINE EMA in febr. 2021 Provided specific obligation to the producer in order to complete post- authorization measure for the conditionate marketing authorization:

Additional information are needed for 1 eccipient ALC-0315 and the syntetic process.

Also as reported in the technical sheet of a m RNA COVID-19 vaccine dec 2021: " 11.1. Information on hazard classes as defined in Regulation (EC) No 1272/2008"

General Information: Toxicological properties have not been thoroughly investigated. The following information is available for the individual ingredients.

11.1. Information on hazard classes as defined in Regulation (EC) No 1272/2008

General Information: Toxicological properties have not been thoroughly investigated. The following information is available for the individual ingredients [9,10].

And related the research works of some reseacher (Campra P, Young RO, Riccardo Benzi C, Young MI Lee et al) [1,2,11] and the methos used before to test and their evidences it is of great interest to match with the EMA sententia that in a written responce confirm that graphene derivates was not present in the sample tested (obserbig the RAMAN spectra in the laboratory of proof related).

So because Graphene derivates are used in many bio technological process due to their properties in absorbtion, extracion, purification, carrier, adiuvant and many other: it is needed to verify the productve process in manifacturing new biopharmaceuticals to verify if impurity are present, what kind and in what concentration. (and also in m RNA COVID-19 vaccine)

All this for toxicological and safety need obviously.

Of interest it is the fact that Scientific literature show various entity in RAMAN -INTENSITY for encapusalted and non encapulates molecule (nanoparticles- liposome).

Direct RAMAN - technique is more efficacy in testing the nanoparticles (and not their payload)

The characteristic kinetic destiny of this nanoparticle during the times it is also of interest: after

Various days the signal increase (disruption of the nanoparticle contribute to make nacked the encapsuled molecule ?) in a reported literature.

So considering all this facts: it is recomended to whom it concern to test as reported in experimental project hypotesys the presence/absence of graphene GO in:

100 vials of the m RNA COVID-19 vaccine - nanolipids using the method of classic analytical chemistry

Like RAMAN destructive method with pre- treatment – extraction of the sample by solvent and 100 vials sample with the method as reported In EP like RAMAN spettroscopy non destructive direct method.

It is needed to send the sample to various certified labs using also control (blinded)

The results must to be collected and the analized in statistical way in order to verify if there is similar results between the two groups or there are significative deviation [9-11].

Conclusion

After this review part, but:

- Related the recent new evidences about graphene derivates finded in some vials of COVID-19 vaccine by independed researcher, that seem not coerent whit the Regulatory agency analitic report and statement,
- The fact that the status of encapsulated molecule show different profile of intensity signal in Raman spettroscop,
- It is stretcly recomend to Perform the experimental hypotesys project submitted using this two methods (classic chemical pre-treatement of the sample before Raman and compared with a NON destructive direct Method as permitted by EP- EMA GMP).
- It is crucial to verify the entity of the nanolipids particles Effect in the Raman Signal of an encapsuled molecule to be searched: it can be relevant for the CQ?

What happen to the signal when dissolved nanolpidis? And nanolipids can influence/reduce intensity of Raman spectra of an analite to be detected?

According the authors only after see this results it will be possible to solve this apparent contraddiction. Between what showed by some independent researcher and the regulatory agency related a same analita. The only way it is to pre-treat the sample in the same way before register Raman for the two groups even if not request by the direct non destructive methods. Finally the entity of this phenomena: reduction of intensity of the signal of the payolad in a nanoparticle What kind of implication can have on GMP - CQ, PAT, regulatory process and for the toxicological Profile of a new innovative biopharmaceutical product? Impurity in classic drugs was observed in some cases even in registered and authorized drugs so why non deeply investigate the impurity profile also of m RNA COVID-19 vaccine? Expecially when some manifacturing procedure are not fully knowed also by regulatory agency and when for some eccipent used the control authority ask to the producer to provide complete information related quality test. It is opinion of the authors that the responce provided by EMA related written question on graphene derivate presence or not in viasl of COVID-19 vaccine must to be integrated with written information

about the Intere analitical process used in the control lab (also related pre-treatement).

Conflict of Interest

None.

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How to cite this article: Luisetto, Mauro, Nili B. Ahmadabadi, K. Edbey Khaled Edbey and L. Cabianca, et al. “Raman Spetroscopy for Biopharmaceutical Quality Control and PAT, Raw Material-Final Products: The Nanolipids Effect on Signal Intensity, Regulatory and Toxicological Aspects.” Pharmaceut Reg Affairs 11 (2022): 328.