White Paper

mRNA Vaccines

UV/Vis Spectrophotometric Analysis

During the outbreak of the coronavirus disease COVID-19, mRNA (messenger RNA) vaccines evolved as efficacious vaccines. Research and development of these vaccines relies chiefly on their quality and equivalent action to a traditional vaccine approach, which can be assessed with advanced analytical techniques.

UV/Vis spectroscopy is one such simple and widely used technique that can help determine the presence and quality of nucleic acids, one of the key components of mRNA vaccines. This document describes promising mRNA vaccines and the potential for UV/Vis spectrophotometry to assist in the accurate and fast characterization of both raw materials and finished products throughout the R&D and manufacturing process.

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METTLER TOLEDO

1. Introduction

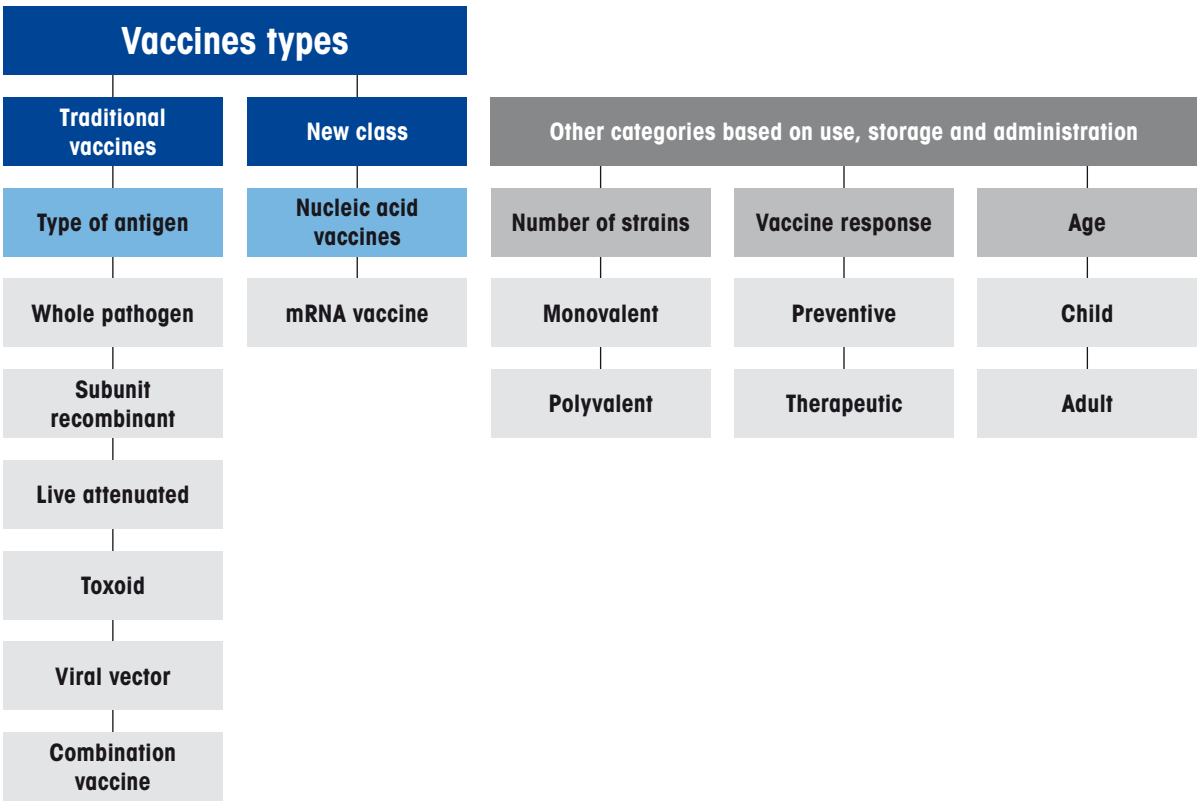
In December 2019, the world learned of a newly discovered coronavirus responsible for COVID-19. Since then, COVID-19, which is scientifically known as Severe Acute Respiratory Syndrome Coronavirus-2 (or SARS-CoV-2), has caused serious illness and loss of life around the world in the form of the COVID-19 pandemic.

Coronaviruses such as COVID-19 comprise a large family of viruses that constantly change through mutation, leading to multiple variants, some of which may have the potential to spread more rapidly. The increasing number of people affected by COVID-19 and the resulting mortality worldwide has necessitated an effective solution to this pandemic in the form of a vaccine.

Vaccine is generally a suspension of weakened, killed or fragmented disease-causing microorganisms. The suspension is administered into the body in a microgram-to-milligram amount in one-to-three small doses [1]. The vaccine then triggers body's immune system, which generates a response that protects the recipient from that particular disease.

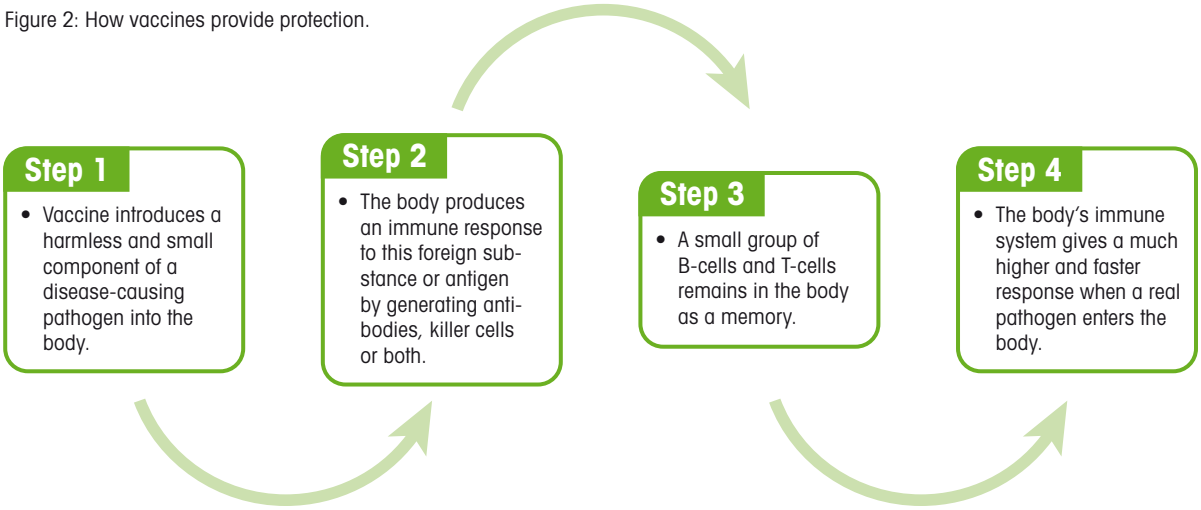
Recently, a new class of vaccine, known as mRNA vaccine, has been added to the list of potential vaccines. This has expanded the categories and nature of available vaccines, which are generally divided into their different classes as shown in Figure 1.

Figure 1: Overview of vaccine types [2].



In all cases, vaccine helps to boost the body's immune system [1]. The common process by which this immune system boost is achieved using vaccines as follows (Figure 2):

Figure 2: How vaccines provide protection.



Researchers across the globe are always working on new vaccines. However, this development process is long and complex. It may take several years to reach a point where a vaccine is determined to be efficacious and safe.

In the case of an emerging disease such as COVID-19, several years may be too long to wait. Therefore, all technologies and processes that can help shorten time-to-availability need to be considered. As you will see in the following pages, UV/Vis spectroscopy is a technology that can provide a solution for characterization of vaccines, their components, and raw materials.

2. Quality Control of Vaccines

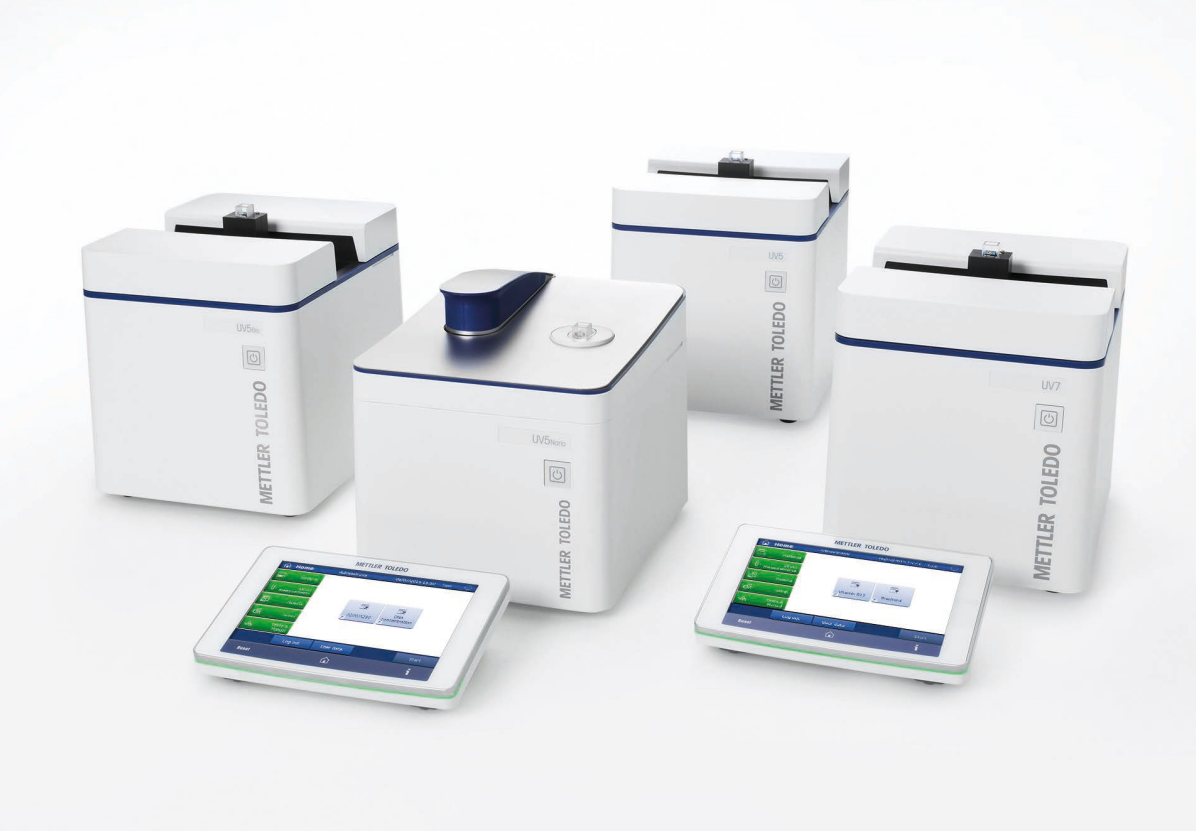
To ensure vaccine safety, it is important to assess quality at every stage of development. The World Health Organization (WHO) along with public health groups such as the National Medical Products Administration (NMPA) China, the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and Japan's Pharmaceuticals and Medical Devices Agency (PMDA) is taking key steps to maintain vaccine quality throughout research and manufacturing. These assessments improve vaccine efficacy and reduce the probability of failure during development and clinical trials.

According to Pfizer, a leading manufacturer of mRNA vaccine, quality control and testing takes more than half of vaccine production time [3]. The legal responsibility to maintain the effectiveness of products at every stage of production generally falls to the manufacturer. Hence, analysis of vaccine raw materials and finished product becomes crucial [4, 5].

Effective mRNA vaccines have been developed for the first time for use against the COVID-19 novel coronavirus and its variants. The development of sensitive, systematic characterization techniques for the virus and its vaccines has been challenging due to the virus' structure, its inherent heterogeneity, and the variety of biological activities it promotes [6].

Among currently available analytical techniques, UV/Vis spectrophotometry is a reliable and convenient tool that can provide information regarding the chemical components of the vaccine at a molecular level. This is important, because e.g., mRNA vaccines require highly purified DNA/RNA to maintain potency. The purity of nucleic acids can be easily checked with the help of UV/Vis spectroscopy.

Figure 3: METTLER TOLEDO UV/VIS Excellence spectrophotometers.



UV/Vis Spectrophotometry

UV/Vis spectrophotometry is a well-established analytical technique used in life science and pharmaceutical laboratories.

Spectrophotometers are popular due to their simplicity and ease of use, as well as the quality of information they provide. The use of these reliable tools has become a standardized analytical method in pharmacopoeias, and they are used daily in analytical laboratories around the world.

3. Nucleic Acid Vaccines and Lipid Nanoparticles (LNPs)

Among current vaccine candidates (see Appendix A), interest has grown in nucleic acid vaccines. These vaccines protect against a disease through use of genetically engineered DNA (as a plasmid) or RNA (as messenger RNA or mRNA).

Compared with other types of vaccines, nucleic acid vaccines contain only synthetically generated DNA or RNA of the virus. They do not contain the live or inactivated virus or any part of it [7]. They are considered next-generation vaccines due to this and other distinct advantages, as shown in Table 1. Potential drawbacks are also presented.

Table 1: Advantages and disadvantages of nucleic acid vaccines.

Advantages	Disadvantages
Relatively easy and rapid synthesis	Long-term side effects are untested
Considered safer, because no live component of the virus is used	Raw materials used such as plasmid DNA and enzymes for capping are either on a limited scale or costly
Do not affect or interact with DNA in the body	Possess low immunogenicity
The cell breaks down the mRNA and gets rid of it soon after it is finished using the instructions	Difficult to ensure effective delivery into the body, because RNA is prone to degradation by nucleases and quickly breaks down
Do not require bacteria or cells for synthesis as they can be produced chemically with the help of a template	Storage conditions are extreme (e.g., ultra-cold storage –70° C)

Essentially, these mRNA vaccines are the most successful candidates for an evolving disease such as COVID-19, showing more than 95% efficacy in preventing serious illness and death [8]. This high efficacy, along with the potential for high potency, rapid development, and safe administration, is causing the mRNA vaccine field to develop expeditiously.

Figure 4: Deoxyribonucleic acid (DNA), the basic building block of life and a primary delivery component of nucleic acid-based vaccines when genetically modified.

The primary obstacle in the development of nucleic acid vaccine historically has been dealing with very delicate genetic material such as RNA. Enzymes in the body break this fragile molecule down very quickly after it enters the body. However, current mRNA vaccine uses lipid nanoparticles (LNPs) as a non-viral vector for better therapeutic delivery.

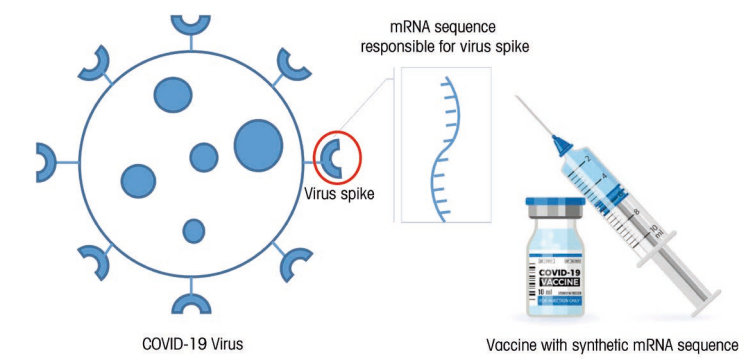
LNPs are tiny balls of fat that envelop and protect mRNA molecules when they are entering the cells. It bypasses the body's guardian mechanism and reaches the target cells (Figure 5). It has been investigated that, without these lipid shells, it is difficult to produce mRNA vaccines for COVID-19 [9]. LNPs are considered less toxic and hence safe when compared to other gene delivery systems, which is why they are the current choice for mRNA vaccines [10].

While the role of LNPs in vaccine development and delivery is still evolving, these tiny particles are thought to be a major reason behind the success of the mRNA vaccine in the prevention of severe COVID-19 disease. Hence, it becomes important to know the working of mRNA lipid nanoparticle assembly in mRNA vaccines.

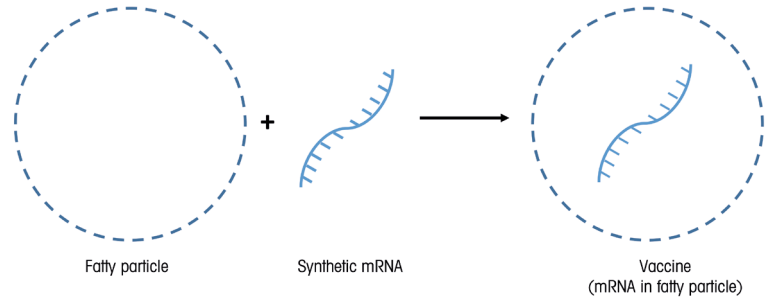
Table 2: Advantages of lipid nanoparticles (LNPs).

Advantages of LNPs
Can be easily synthesized compared to other gene-delivery vehicles
Effective as carrier vehicles
Protects RNA (mRNA) molecules from degradation
Possesses potential to deliver therapeutics by escaping the entrapment of guardian pathways of the body such as endo/lysosomal compartments
Can be co-delivered with adjuvants
Biocompatible and biodegradable nature

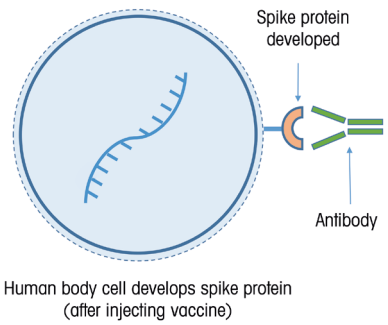
Figure 5: How the COVID-19 mRNA vaccine provides protection.



- The virus contains spike protein and a specific mRNA sequence.
- mRNA vaccine contains synthetically created mRNA having the same sequence as that of the virus.



- mRNA enters the muscle cell at the injection site.
- mRNA can quickly degrade in the body, hence, they are packed inside a very small fatty particle.



- mRNA delivers genetic code to cells and instructs them to produce a coronavirus surface protein or spike protein themselves.
- The body's defense system recognizes the spike protein as a foreign entity and begins to protect itself by developing antibodies to it.

4. mRNA-LNPs Vaccine Manufacturing for COVID-19

On December 29, 2020, the WHO released a report on several potential RNA-based COVID-19 vaccines that are in different stages of clinical trials. Two candidates, Moderna and Pfizer-BioNTech, received emergency use authorization and showed remarkable results [11, 12].

General synthesis of **mRNA vaccines** is straightforward:

mRNA + Lipids + Other + Buffers → RNA vaccine Finished product

Four types of lipids are primarily used in mRNA synthesis. These are cationic/ionizable lipids (for encapsulation), anionic/neutral lipids (for fusogenicity), PEG lipids (for stability, immunogenicity) and cholesterol (for stability). Other components such as sucrose are added to maintain the shape and stability of vaccine during freezing. Buffers are used for final storage and stability.

The formulation of the **Moderna mRNA vaccine:**

mRNA + Cholesterol, DSPC, SM-102, PEG2000-DMG (Lipids) + Sucrose + Buffers → mRNA-1273

Buffer components for the Moderna vaccine are TRIS, TRIS-HCl, acetic acid, sodium acetate and water [12].

The formulation of the **Pfizer-BioNTech mRNA vaccine:**

mRNA + Cholesterol, DSPC, ALC-3015, ALC-0159 (Lipids) + Sucrose + Buffers → Comirnaty or BNT162b2

Buffer components for the Pfizer-BioNTech vaccine are potassium chloride, monobasic potassium phosphate, sodium chloride, basic sodium phosphate dehydrate and water [13].

As per the Pfizer-BioNTech detailed manufacturing process:

- Specially designed E. coli. bacteria undergo the process of photosynthesis and produce DNA plasmids in large quantities.
- This solution undergoes a purification process to remove everything except DNA plasmids.
- DNA loops are then linearized with the help of enzymes. Here, high-quality DNA lines are transcribed into messenger RNA, i.e., mRNA, using enzymes.
- Samples are tested at every stage during development to maintain quality standards.
- Furthermore, fragile mRNA strands are encapsulated in LNPs to make them stable in nature.
- Lipids are first diluted with ethanol and then mixed with mRNA to get the final vaccine product that is then filled into vials [3].

Before coming into the market, vaccines must pass stringent regulatory procedures and each lot must be tested for quality. Regulatory agencies such as FDA work along with the manufacturer to develop and release a protocol for vaccine manufacturing and testing [13].

For liposome-based drug products including mRNA vaccines, it is necessary to perform analytical tests supported by spectroscopic or other analytical methods [14] in order to assess parameters that affect purity, shelf-life, efficacy, and more.

5. Usage of UV/Vis Spectrophotometer in Quality Control of mRNA Vaccines

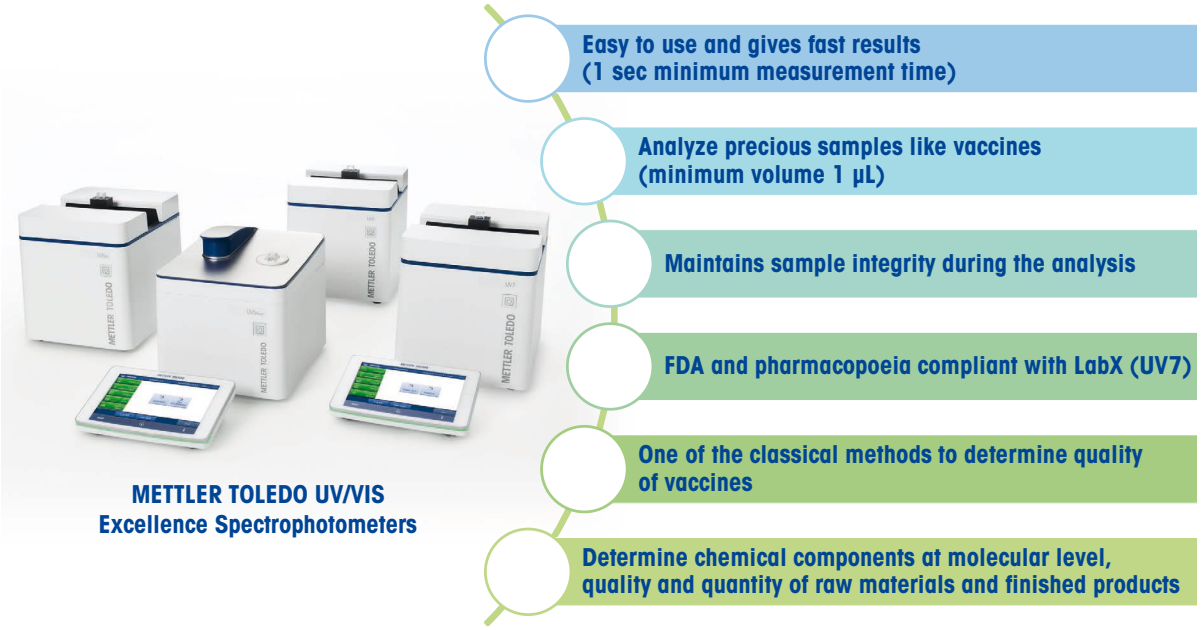
The nature and composition of mRNA vaccines make them inherently difficult to characterize. Characterization of vaccine components is also a challenge in R&D, pilot production, manufacturing, quality control, and control pre- and post-sale.

UV/Vis spectrophotometric determination is considered a standard method for determination of nucleic acid content. In addition, it can be used in the analysis of bacteria, enzymes, proteins, nucleotides, plasmids, as well as for purity/impurity profiles of raw materials such as ethanol and sucrose. This flexibility along with ease of analysis has made it an analysis of choice for characterization of both final vaccines and various vaccine components across the development chain.

COVID-19 vaccine manufacturers and quality control departments are sharing information about the analytical techniques that are helping them to standardize vaccine quality [8]. According to international pharmacopoeias including the European Pharmacopoeia (Ph. Eur.), United States Pharmacopoeia (USP), Japanese Pharmacopoeia, and the British Pharmacopoeia, UV/Vis spectrometry is a classical method for physicochemical characterization of vaccines that is being put to use in COVID-19 manufacturing research [16, 17].

The METTLER TOLEDO UV/VIS Excellence spectrophotometer series (UV5, UV7, UV5Bio and UV5Nano) can assist with in the synthesis and characterization of vaccine and raw materials. The advantages of METTLER TOLEDO UV/VIS Excellence spectrophotometers are as follows:

Figure 6: Properties of METTLER TOLEDO UV/VIS Excellence series spectrophotometers.



UV/VIS Excellence spectrophotometers can determine all chemical components of a vaccine or vaccine raw material if:

- The sample absorbs or transmits distinctly within the UV or Vis region
- (spectral range 190–1100 nm)
- The sample is a clear and transparent liquid or a transparent creaseless solid
- The sample is turbid but needs to be measured for its absorbance

6. Possible Characterization Areas for UV/Vis Spectroscopy

With the expansion of interest in mRNA vaccines, various analytical techniques including UV/Vis spectrophotometry can be used in the characterization and analysis of these vaccines and their raw materials. An overview of vaccine development areas where UV/Vis spectroscopy can help to improve speed and accuracy is mentioned in Table 3.

Table 3: Areas of use for METTLER TOLEDO UV/VIS Excellence spectrophotometer in mRNA vaccine development and manufacturing during COVID-19 pandemic.

Parameters	UV/Vis spectrophotometric characterization areas	Raw materials	Finished product	Wavelength	Suggested Instrument
Physiochemical properties	• Appearance / clarity of finished product [18] • Degree of coloration of liquids [18, 19] • Stability studies (spectroscopic profiles) [18, 20] • Bacterial growth rate [21] • Microbial quality [18]	✓	✓	Sample specific 400–700 nm Sample specific 200–900 nm 420–615 nm	UV5 / UV7 UV5Bio
Nucleic acids	• DNA/RNA concentration, purity, quantity [22, 23, 24] • Quality of oligonucleotides, nucleic acid denaturation [23]	✓	✓	260 nm – strong absorbance	UV5Nano
Proteins	• Quantification of proteins [24] • Absorption coefficient of proteins, protein unfolding, protein contamination in nucleic acid [23]	✓	✓	UV region ~180–300 nm 820 nm	UV5Nano UV5Bio
Enzymes	• Enzyme activity, enzyme kinetics, e.g., lipase enzyme [23, 24, 25]	✓	–		
Chemical components	• Components in DNA, e.g., phosphorus, phosphates [26, 27]	✓	–	820 nm	UV5 / UV7
Sucrose	• Quantitative and qualitative determination [28]	✓	✓	340 nm	UV5 / UV7
Lipids	• Total lipids [29] • Purity, concentration of phospholipids in liposomes [30, 31] • Identification and extraction kinetics of lipids [32] • Oxidation of phospholipids [33] • Phase transition [34] • Cholesterol (total cholesterol) [35]	✓	✓	675 nm Sample specific 190–300 nm 234 nm Sample specific 570 nm	UV5 / UV7
Ethanol	• Purity of ethanol [36, 37] • Concentration of ethanol [38]	✓	✓	235–340 nm 340 nm	UV5 / UV7
Raw materials	• Analysis fatty acids [39]	✓	–	Sample specific	UV5 / UV7
PEG	• PEG Molecular weight [40] • PEG concentration [41]	✓	–		
Lipid nanoparticles (LNPs)	• Turbidity for size determination NPs [42] • Turbidity for agglomeration, stability, aggregation, changes in surface plasmon resonance [42, 43, 44, 45] • In understanding structure of mRNA LNPs [46]	✓	–	Sample specific	UV5 / UV7
Bacterial endotoxins	• Presence of endotoxins in pharmaceutical products by turbidimetric technique, kinetic-turbidimetric assay [47, 48]	✓	–	340 nm	UV5 / UV7
Buffer	• Purity and quantity of acetic acid [49]	✓	✓	340 nm	UV5 / UV7
Miscellaneous	• Components present in serum of volunteers during clinical trials for [50, 51, 52] • Potassium (K) [52] • Glucose [53] • Blood urea nitrogen (BUN) [54] • Creatinine [55] • Calcium (Ca) [52] • Magnesium (Mg) [56] • Cholesterol [35] • Enzymes [25] • Heamoglobin (Hb) [57]	–	–	690 nm 340 nm 420 nm 400–700 nm 550 nm 520 nm 500 nm Sample specific 590 nm	UV5 / UV7 UV5Bio
	• Medical wastewater, e.g., residual chlorine [52, 58]	–	✓	550 nm	UV5 / UV7

Note

- Wavelength for determination and suggested instrument may vary depending upon the procedure or method chosen for the analysis.
- Turbidity can be measured with METLLER TOLEDO UV/VIS Excellence Spectrophotometers but up to certain limit (refer to Table 4 for turbidity application)
- LNPs are cloudy in nature, due to which light scattering may alter the results if measured beyond the detection limit of the instrument.
- UV7 with hardware version 2 and firmware 3.0.0 onwards is ensured to comply with US and Eur. Pharmacopoeia requirements.
- The above references in Table 3 are based on existing literature. It can be revised and updated based on new trends and ongoing research in the mRNA vaccine development.

7. Supporting Application Notes and Literature

Table 4: Published literature applicable to COVID-19 and mRNA vaccines research referencing METTLER TOLEDO instrument use.

Documents	
Application note	**Sucrose** www.mt.com/in/en/home/supportive_content/ana_chem_applications/uvvis/M9004.html
Application note	**DNA/RNA: Purity and Concentration of Nucleic Acids** www.mt.com/in/en/home/supportive_content/ana_chem_applications/uvvis/M9501.html
Application note	**Impurities in Ethanol** www.mt.com/in/en/home/supportive_content/ana_chem_applications/uvvis/M9307.html
Application note	**E. coli.: OD600 of Escherichia coli.** www.mt.com/in/en/home/supportive_content/ana_chem_applications/uvvis/M9504.html
Application note	**Enzyme Activity** www.mt.com/in/en/home/supportive_content/ana_chem_applications/uvvis/M9601.html
Application note	**Formazin Turbidity** www.mt.com/in/en/home/supportive_content/ana_chem_applications/uvvis/M9601.html
White paper	**UV/Vis Applications in COVID-19 Vaccine Research** www.mt.com/in/en/home/library/white-papers/lab-analytical-instruments/spectrophotometric-covid19-vaccine-analysis.html
Poster	**Nucleic Acid Analysis with Absorbance Spectroscopy** www.mt.com/in/en/home/library/tips-and-tricks/lab-analytical-instruments/nucleic-acid-analysis.html
Document	**UV/Vis Life Science Applications** www.mt.com/in/en/home/library/guides/laboratory-division/life-science/uvvis-toolbox-for-life-sciences.html

8. Glossary

ALC-0159	(2-hexyldecanoate),2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
ALC-3015	(4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis
BNT162b2	COVID-19 vaccine from Pfizer Inc./BioNTech Inc.
COVID-19	Coronavirus Disease 2019
DNA	Deoxyribonucleic acid
DSPC	1,2-distearoyl-snglycero-3-phosphocholine
EMPA	European Medicines Agency
LNPs	Lipid Nanoparticles
mRNA	Messenger Ribonucleic acid
mRNA-1273	COVID-19 vaccine by ModernaTX Inc.
NMPA	National Medical Products Administration
PEG	Polyethylene glycol
Ph1	Phase 1
PMDA	Pharmaceuticals and Medical Devices Agency
RNA	Ribonucleic acid
R&D	Research and Development
RSV	Respiratory syncytial virus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
SM-102	(heptadecan-9-yl 8-((2-hydroxyethyl) (6-oxo-6-(undecyloxy)hexyl)amino) octanoate)
TRIS	Tromethamine
TRIS-HCl	Tromethamine hydrochloride
US FDA	United States Food and Drug Administration
UV/VIS	Ultra-Violet/Visible
WHO	World Health Organization

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Appendix A: Current COVID-19 Vaccine Options

Over the years, several vaccine platforms have been developed to treat diseases. Among them, four primary types, listed in Figure 7 that follows, are currently approved, in the process of being approved, or are in use as effective therapy against developing an active or severe case of COVID-19 [59].

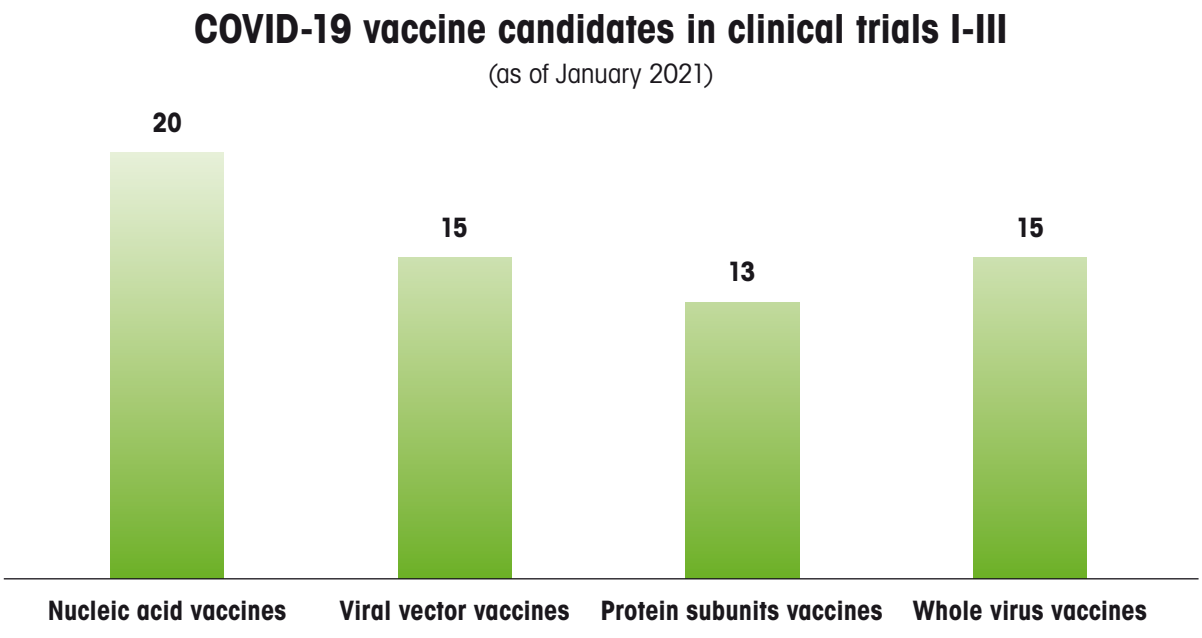
Figure 7: Four types of COVID-19 vaccines.

Nucleic acid vaccines	Viral vector vaccines	Whole virus vaccines	Protein subunit
• Uses genetic material – either RNA or DNA – to provide cells with the instructions to make the antigen • **Vaccines include:** Pfizer-BioNTech, Moderna	• Uses a harmless virus to give the cells genetic instructions to produce antigens • **Vaccines include:** AstraZeneca, Sputnik V, Covaxin	• Uses whole viruses (live attenuated or inactivated) to trigger an immune response • **Vaccines include:** Sinopharm, Sinovac	• Uses pieces of the pathogen – often fragments of protein – to trigger an immune response • **Vaccines include:** Novavax

There are approximately 184 COVID-19 vaccines in preclinical trials, 35 vaccines in phase 1 study, 34 vaccines in phase 2 study, 28 in phase 3 trials, and 17 vaccines in use (as of July 2021) [60]. These numbers continuously expanding with the increase in demand for vaccines.

The research on nucleic acid vaccine candidates (especially mRNA vaccines) has gained in popularity, as is shown in the Figure 8 that follows.

Figure 8: Progress of COVID-19 vaccine candidates [59].



Appendix B: mRNA Vaccines beyond COVID-19

mRNA vaccines for COVID-19 are the first mRNA vaccines to be developed, reach stage 3 trials, and become licensed for use in humans [60]. Several manufacturers have achieved exceptional success in the race to develop effective COVID-19 vaccines [62].

Although mRNA vaccines are rolled out worldwide for COVID-19, additional mRNA vaccines are now in development to prevent and treat other potentially fatal infections beyond COVID-19, e.g. influenza, which is constantly moving and mutating.

Table 5: mRNA vaccines being studied in infectious diseases beyond COVID-19 [61].

Disease target	Study stage	mRNA	Delivery formulation	Organization
RSV	Ph1, ongoing	mRNA-1172	Merck proprietary formulation	Merck/Moderna
Influenza (H10N8)	Ph1, ongoing	mRNA-1140	LNP	Moderna
Zika virus	Ph1, completed	mRNA-1325	Not disclosed	Moderna
Rabies	Ph1, completed	CV7201	Protamine	CureVac
Chikungunya virus	Ph1, ongoing	mRNA-1388	Not disclosed	Moderna

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