Review Article

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Gamma irradiation-mediated inactivation of enveloped viruses with conservation of genome integrity: Potential application for SARS-CoV-2 inactivated vaccine development

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Abstract: Radiation inactivation of enveloped viruses occurs as the result of damages at the molecular level of their genome. The rapidly emerging and ongoing coronavirus disease 2019 (COVID-19) pneumonia pandemic prompted by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is now a global health crisis and an economic devastation. The readiness of an active and safe vaccine against the COVID-19 has become a race against time in this unqualified global panic caused by this pandemic. In this review, which we hope will be helpful in the current situation of COVID-19, we analyze the potential use of γ-irradiation to inactivate this virus by damaging at the molecular level its genetic material. This inactivation is a vital step towards the design and development of an urgently needed, effective vaccine against this disease.

Keywords: SARS-CoV-2 virus, virus genome, virus infectivity, gamma irradiation, virus inactivation, vaccine development

1 Introduction

Viruses (or virions) are subcellular particles, commonly spherical or rod-shaped, which composed of a protein capsid that contains their genetic material made of RNA or DNA. Sometimes the viral genome is protected by an additional outer envelope made of a lipid bilayer with spikes of glycoproteins inserted inside the viral envelope [1]. Viruses are classified based on their size, shape, envelope, and structure of their genome. Unlike bacteria, viruses lack cell organelles and thus have no metabolic activities on their own. To transcript and replicate, they entirely depend on the host biochemical machinery of eukaryotic or prokaryotic host cells [2]. Once inside the host cell, viruses can mutate through genome deletion, insertion, and/or substitution to novel strains of different virulence [3,4]. This viral mutation is the major obstacle for the development of new vaccines [5,6].

1.1 Key features of human coronavirus

Six human coronaviruses (HCoVs) were known before the COVID-19 outbreak: 229E and NL63 (alpha coronavirus), OC43, HKU1, SARS-CoV, and MERS-CoV (beta coronavirus) [7]. Severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) first emerged in South China in 2002–2003 to cause a large-scale epidemic with over 8,000 infections and more than 800 deaths [8]. The Middle East Respiratory Syndrome CoV (MERS-CoV) has caused a persistent epidemic in the Arabian Peninsula, especially in Saudi Arabia in 2012 [9]. SARS-CoV and MERS-CoV are enveloped positive-sense RNA viruses (size ranging from 70 to 90 nm) belonging to the Coronaviridae family. It was shown that rodents, avians, and mainly bats are reservoir host of these family viruses that can be potentially transmitted from animals to humans [10,11] due to the growing consumption of animal proteins including those from exotic wild mammals in China.

A novel strain of coronavirus, labeled as SARS-CoV-2 by the International Committee on Taxonomy of Viruses-Coronavirus Study Group [12], belonging to the beta-coronavirus lineage, shares around 80% identity to SARS-CoV [13,14]. This strain, believed to have started

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from a seafood market in the city of Wuhan, China, in December 2019, is now spreading and creating a chaos across the entire world [15,16]. Human-to-human transmission of this virus was officially confirmed on January 20th 2020 [10]. World Health Organization (WHO) declared this disease as a global pandemic on March 11, 2020 [17]. As of 29 January 2021, 27,901,760 cases are still active, 72,337,017 recovered, and 2,210,259 died in 235 countries or territories worldwide [18].

Similar to previous coronaviruses symptoms, SARS-CoV-2 is mainly affecting the lower respiratory track, ranging from mild respiratory disease to SARS and septic shock in advanced stages. Damages to the cardiovascular system, gut, kidneys, and brain have also been reported [13,19] along with vital organ failures in comorbid patients [20,21].

The genetic material of SARS-CoV-2 consists of single-stranded RNA backbone made of alternating 5-carbon sugar (ribose) and phosphate groups. Attached to this backbone are 29,891 to 29,903 adenine, uracil, cytosine, and guanine bases [22,23] encoding for 9,860 amino acids [10]. One third of the SARS-CoV-2 genome make up the four major structural proteins: spike glycoprotein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) accountable for some important functions in virus replication [24,25]. The remaining two-thirds of its viral genome encode for 16 nonstructural proteins (nsp-1 to 16). Each of these nsps has a specific role in the life cycle of the virus and its pathogenicity [26,27]. For instance, nsp-1 is used by the virus to elude the host innate immune system [28], nsp-2 is indispensable for its replication, and nsp-9, in complex with nsp-8, is involved in RNA replication and virulence [29].

There is a 76.5% similarity in the amino acid sequences of the spike glycoprotein in SARS-CoV and SARS-CoV-2 [30]. SARS-CoV-2 seems to have greater binding affinity to the angiotensin converting enzyme 2 (ACE2) cell membrane receptor than the other SARS-CoV virus strains, suggesting a greater capacity of SARS-CoV-2 for human to human transmission [31,32]. There are speculations that cellular overexpression of human ACE2 (associated with the usage of medications such as ACE2 inhibitors, angiotensin II receptor blockers) could enhance the COVID-19 severity [21,33–36]. Among the four structural proteins of the SARS-CoV family, the spike glycoprotein S plays a key role in viral docking and cellular internalization [37]. It binds via the receptor-binding domain (RBD) in the S1 subunit to the ACE2 receptors [38,39], expressed especially on the plasma membrane of human respiratory epithelial host cells, and virtually in all other organs. The virus penetrates the host cell via endocytosis through its S2 subunit [40,41] and infects it by hijacking its molecular machinery to encode for RNA polymerase enzyme necessary for the replication of its own RNA genome [42,43]. Viral entry in cells is a critical phase in the course of the COVID-19 disease. Thus, inhibition of the viral binding and internalization in a host cell constitute a strategy for potential therapeutics against COVID-19 pandemic.

1.2 SARS-CoV-2 infectivity

Aerial transmission by expelled respiratory droplets is considered as the main direct transmission vector of the SARS-CoV-2 when in close contact with an infected person coughing, sneezing, or even talking [10,44–46]. Some findings have indicated that the virus may as well be airborne [31,47,48]. Indirect transmission may also occur via fomites when respiratory droplets from infected people land on object surfaces which can be touched by a receptive host [49,50].

The WHO recommends that SARS CoV-2 sample handling should be conducted in no less than a Biosafety Level 3 (BSL-3) laboratory using BSL-3 practices (WHO, 2020) [17,51]. The SARS-CoV-2 cytopathogenic effect is measured by its capability to infect a host cell. This is usually expressed by the tissue culture infectious dose, $TCID_{50/mL}$, calculated using the method of Reed and Muench, [52], which is the viral titer at which 50% of the host cell lines are infected when inoculated *in vitro* with a diluted viral solution. However, because of some limitations with the *in vitro* tests (slow viral growth), the use of *in vivo* assays is taken after inoculated animals were sacrificed for within-host virus titering and pathological study [53].

Basic reproductive number R_0 is a key epidemiological factor used to measure the potential infectivity of virus-related outbreak [54,55]. It represents the average number of infected people caused by one infected individual during his/her whole contagious phase. Values of R_0 less than one means that the pandemic is most likely to stop propagating. In early stage of COVID-19 pandemic, realistic pooled values of R_0 were estimated in 29 studies, all done in China, using different mathematical methods [56]. The mean value of R_0 was evaluated as 3.38 ± 1.40 (95% confidence interval 1.9–6.49). R_0 was found to be between 2.43 to 3.10 in Italy in early stage of COVID-19 pandemic [54].

One of the key epidemiological factors in the COVID-19 pandemic is the incubation period, time elapsed between the exposure and the appearance of the first symptoms. Different pooled analysis of confirmed COVID-19 cases showed that the estimated incubation times were 5.1 days [57], 6.4 days (range 2.2–11.1) [58], and 5.0 days (range 2–14 days) [59]. Sanche and colleagues estimated, in late January 2020 in Mainland China, the average incubation period to 4.2 days. A time duration from symptoms onset to admission to hospital for treatment was estimated to

1.5 days in late January 2020 and the time from symptoms onset to death to 16.1 days [60].

Various *in vitro* studies showed that RNA viruses are less vulnerable to corruption due to their ability to promptly repair their genome damages by proof-reading, excision, and removing flawed RNA nucleotides that occur during their replication [61–64]. Exoribonuclease (ExoN) enzymes encoded by nsp-14 play a crucial role in maintaining the viral genome integrity [18,64–66]. Eckerle and workers reported that mutations in nsp-14 of SARS viruses lead to 15-fold increase in replication errors [67].

1.3 Vaccine strategy

To help prevent the spreading of the COVID-19 pandemic, most of the countries have adopted immediate measures consisting of global travel restrictions on movement, lockdowns, social distancing, patient self-isolation, and provision of medical care to infected people. Few curative methods using already known antiviral agents such as hydroxychloroquine and remdesivir were tested on patients [68–70]. However, results were not very encouraging for any of these agents to be considered as significant therapy yet. The best option for ending this pandemic and reestablishing a normal life remains, by far, the development of safe and effective prophylactic vaccines. This has triggered an extensive collaboration and a colossal mission between pharma companies and scientists to expedite vaccine development and production in less than a year instead of the normal 10-year period time. By the end of 2020, 259 COVID-19 vaccine projects were in the pipeline [71]. Frontrunning coronavirus vaccines, sharing the same purpose of stimulating the immune system against SARS-CoV-2, can be broadly categorized into three platforms:

- the classical inactivated virus vectored vaccines based on disrupting the viral genome through chemical or physical alterations. These viruses are no longer able to replicate to cause infection, but able to trigger an immune memory response [72].
- the full-length S glycoprotein- or RBD-based vaccines that generate target antigens in the infected cell [73].
- the groundbreaking DNA-, mRNA-based vaccines that encode in the host cell the full-length S glycoprotein as target antigen [74].

As of 29 January 2021, five vaccines went through the necessary multiple phases of trial to ensure safety, showing more than 90% efficacy. They have been approved and licensed for use by national and international public health regulators and are being rolled out worldwide (Table 1).

1.4 Different agents for SARS-CoV-2 inactivation

Virus inactivation for vaccine purposes was already known since the late 1800s [84]. In 1885, Pasteur laid the foundations of immunization with inactivated rabies virus cultured in rabbit spinal cords [85]. It was not until the discovery of the *in vitro* culture of viruses outside the host organism procedures that inactivated viral vaccine development was truly initiated. This allowed a large-scale production of viruses as source for inactivated vaccine purposes [86]. Vaccine producers are generally using virus growth on continuous cell lines to reduce production costs and increase vaccine safety. Once the virus has been purified, inactivation can be achieved using chemical or physical methods or a combination of the two. A wide range of chemical agents are used: ascorbic acid [87], derivatives of ethylenimine [88], and hydrogen peroxide [89]. However, formaldehyde [90] and β-propiolactone [91] are the most widely used for inactivation for decades. To avoid the extensive and time-consuming downstream processing to detoxify the virus cultures from chemical inactivators, the use of γ-irradiation as a physical alternative

Table 1: COVID-19 vaccines currently available in the market (January 2021)

Vaccine	Platform	Inactivation method	Developer	Reference
BNT162b2	mRNA	—	Pfizer – BioNTech (USA, Germany)	[75,76]
mRNA-1273	mRNA	—	Moderna (USA)	[77,78]
AZD1222	Nonreplicating viral vector	Deletions in E1 and E3 genes in adenovirus vector to inhibit replications	University of Oxford AstraZeneca (UK)	[15,79,80]
CoronaVac	Inactivated SARS-CoV-2	β-Propiolactone to inhibit replication	Sinovac (China)	[29,81]
Sputnik V	Heterologous recombinant adenovirus (rAd26 and rAd5)	Deletions in E1 and E3 genes in adenovirus vector to inhibit replications	Gamaleya (Russia)	[82,83]

method to chemical inactivation has been proposed by many authors. Preparation of experimental vaccines against several viral diseases using γ-irradiation is reported in the literature: bluetongue [92], Venezuelan equine encephalitis [93], rabies [94], smallpox [95], influenza [96], HIV [97], Ebola [98], rotavirus [99], and polio [100].

The choice of an inactivation method preserving the viral epitope integrity is important since the damage of the envelop protein will lessen the efficacy of the vaccine [101]. Several studies showed that viral inactivation by formaldehyde, hydrogen peroxide, or binary ethylenimine derivatives is nonselective and can damage the envelope protein leading to a poor immune response [102,103]. Nevertheless, γ-irradiation has shown a superior inactivation method by preserving the viral antigens intact to trigger the immunogenicity while destroying nucleic acids to inhibit the viral replication in human cells [104,105]. This advantageous attribute of γ-irradiation can be ascribed to its high penetration depth that causes direct damage to nucleic acids without altering structural proteins [96,106,107].

Due to the potentially dangerous consequences of SARS-CoV-2 human infection, extreme attention should be paid to ensure that inactivation procedures are efficient. Effective inactivation of the SARS-CoV-2 is vital as it allows research, especially the development of new vaccines, to be conducted under safe conditions [108]. Various methods are already available for SARS-CoV effective inactivation [109] and could be tested on SARS-CoV-2 since these two viruses share a great deal of genome: ultraviolet radiation, thermal treatment, extreme pH values, and commonly used disinfectants offer an effective virus inactivation. Duan and colleagues reported an inactivation of SARS-CoV virus after 15 min exposure to ultraviolet C, whereas ultraviolet A and B had no effect on its viability, irrespective of the duration of the exposure [110]. Heat can denature SARS-CoV secondary structural proteins. A complete inactivation of this virus at 75°C for 45 min was reported by Darnell and colleagues [111]. For inactivation with detergents, Gerlach et al. showed that SARS-CoV-2 can be efficiently inactivated by 70% ethanol, 0.1% hydrogen peroxide, and 0.1% sodium sulphate, commonly available in hand soaps, within 60 s of exposure on various surfaces [112]. pH has a great effect on the viability of SARS-CoV-2. Chan et al. reported that the virus survived for up to 6 days in a medium with pH range [5–9], but lost between 2.9 and 5.33 $\log_{10}$ infectivity. At pH 4 and pH 11, it remained viable for 1–2 days. At extreme pHs (pH 2–3 and pH 11–12), the virus lost 5.25 $\log_{10}$ infectivity within only 1 day [16].

In this perspective, γ-inactivation of viruses could be an important and promising tool for SARS-CoV-2 vaccine development (Figure 1). In this review, we analyze the potential use of γ-irradiation to inactivate the SARS-CoV-2 by altering its genetic material while preserving its structural proteins.

1.5 Radiolysis of water in biological matter: mechanisms of radiobiological action

The two major processes in the interaction of energetic photons or charged particles with aqueous biological medium are ionization and excitation of water molecules and biologically important macromolecules such as DNA, RNA, lipids, and proteins [113,114]. The electrons liberated in the ionization have enough energy to ionize further

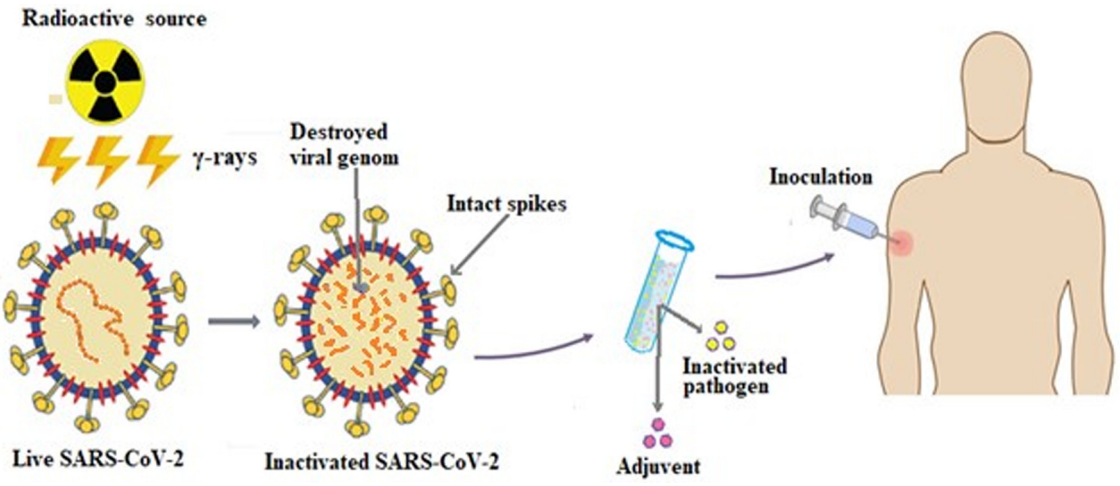


Figure 1: Schematic diagram showing the development of SARS-CoV-2 vaccine using radiation-induced inactivation of live virus.

molecules in a manner that energy is deposited in spurs. When their energy falls below the ionization threshold of water, the electrons become solvated e^-_{aq}. Excited water molecules can dissociate into free radicals $OH^\bullet$ and $H^\bullet$. These physicochemical processes, concentrated in tracks along the path of the ionizing species, are complete approximately 10^{-12} s after the absorption of the ionizing radiation [115,116]. The radiolysis products either react with each other within the spurs to produce reactive oxygen species (ROS), such as superoxide radical $O_2^{\bullet-}$ and its conjugate perhydroxyl radical $HO_2^\bullet$, or diffuse homogeneously into the bulk of the biological medium where they are scavenged by biological macromolecules. The spur diffusion is complete approximately 10^{-7} s after the absorption of the ionizing radiation [117].

1.6 Mechanism of viral inactivation

γ-irradiation disrupts viruses by altering mainly their RNA genetic material. The number of nitrogenous bases and their sequence in the RNA is crucial for determining the viral sensitivity towards γ-irradiation. The more target nucleotides, the more likely the nucleic acid genome will be damaged for a given absorbed dose [118]. The mechanism behind this damage falls into two types: direct and indirect. Direct damage is caused by the radiation-induced cleavage of the sugar-phosphate backbone or the cross-linking, deletion, substitution, and insertion in the sequence of nitrogen bases [119,120]. Indirect damage is attributed to the oxidative stress of the radiolytically produced ROS on the viral material, leading to its fragmentation and cross-linking [121,122]. A minimum energy deposition of 17.5 eV within a critical distance of 6 Å from the nucleotide (corresponding to a sensitive spherical volume of 0.596 nm^3) can induce a lethal damage to the viral RNA [123]. Disruption to the protein capsid and the lipid bilayer envelope, by lipid and protein peroxidation chain reactions, may as well result in the reduction of viral pathogenicity [70,124,125]. It has been reported that, for viruses belonging to the enveloped Coronaviridae family, the conformational changes in the spike glycoprotein S block the viral binding to the host cell plasma membrane and prevent cellular internalization, the first stage in viral infection [111,126]. Studies suggested that genetic material rather than protein and lipid envelopes is likely to be the primary target for viral inactivation [107,127]. Nims et al. reported that the presence or absence of a viral envelope does not seem to be a major factor of inactivation by γ-irradiation [128].

Single-strand break (for single-stranded viruses) and double-strand break (for double-stranded viruses) are generally sufficient to inactivate the viral genome [129]. Based on the hypothesis of the single-hit-single-target (SHST) model [130,131], the inactivation of viruses is typically expressed by the following relationship [132]:

$$N(D) = N_0\, 10^{-\frac{D}{D_{10}}} \tag{1}$$

where N_0 and $N(D)$ are the virus population before and after the irradiation, respectively, D being the radiation dose. D_{10} depicts the required irradiation dose to reduce the initial virus titer by 90% (or reduce the population by a factor of 10). D_{10} values vary between different types of viruses mainly due to the significant differences in their genome, capsid morphology, and the presence or absence of an envelope. For convenience, the viral inactivation is expressed in terms of $\log_{10}$ reduction, which is the logarithm base 10 of the ratio of the viral titer before (N_0) and after ($N(D)$) the inactivation:

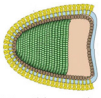
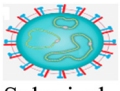
$$\log_{10}\ \text{reduction} = \log_{10}\left(\frac{N_0}{N(D)}\right) \tag{2}$$

It should be noted that D_{10} corresponds to 1 $\log_{10}$. For instance, 1 $\log_{10}$ reduction corresponds to 90% reduction (or 10-fold) and 2 $\log_{10}$ reduction corresponds to 99% reduction (or 100-fold).

Feldmann and colleagues showed that the inactivation was inversely correlated with genome size [133]. They measured the radiation doses for a 6 $\log_{10}$ reduction and found 2 Mrads for coronaviruses (~29 kb genome size), 4 Mrads for filoviruses (~19 kb), 8 Mrads for arenaviruses, bunyaviruses, orthomyxoviruses, and paramyxoviruses (~13 kb) and 10 Mrads for flaviviruses (~9 kb). Viruses having single-strand nucleic acid present the highest radiosensitivity. Hume and colleagues [127] reported that the three enveloped single-stranded RNA viruses of similar sizes, namely morbillivirus (90–150 nm), bunyavirus (90–120 nm), and rhabdovirus (70–150 nm), showed a comparable D_{10} values (2.53, 2.61, and 2.71 kGy respectively) when irradiated under the same experimental protocol.

Leung and workers cultured SARS-CoV-2 on Vero cells in the presence of 1% fetal bovine serum and 1% L-glutamine. Virus-containing supernatants were titered after irradiation [134]. The D_{10} was 1.6 kGy and the complete inactivation of the SARS-CoV-2 was attained with an absorbed dose of 10 kGy, value lower than the 20 kGy previously reported value for the similar SARS-CoV [133]. Even though the single-stranded RNA viruses may present a certain radioresistance, Nims and colleagues showed that no strong clue can explain the discrepancies in the

Table 2: Viral properties of some enveloped virus families. Efficacy of γ-irradiation on the $\log_{10}$ reduction

Virus	Family	Morphology (not to scale)	Size (nm)	Genome size (kb)*	Nucleic acid genome	D_{10} (kGy)	$\log_{10}$ reduction/ kGy[c]	References
IBR[b]	Herpesviridae	 Spherical	100–120	120–230	Double-stranded DNA	3.22	0.310	[136,137]
APV	Poxviridae	 Oval	240–300	130–260	Double-stranded DNA	2.20	0.456	[138,139]
PI3[b]	Paramyxoviridae	 Spherical	100–200	13–19	Single-stranded RNA	4.78	0.209	[128,140]
BVDV[a]	Flaviviridae	 Icosahedral	50–70	9–13	Single-stranded RNA	5.05	0.198	[141,142]
SARS-CoV-2	Coronaviridae	 Spherical	20–25	26–32	Single-stranded RNA	1.60	0.625	[134,143]
BEFV[a]	Rhabdoviridae	 Bullet shaped	75 × 150	10–16	Single-stranded RNA	2.94	0.340	[142,144]
Akabane[a]	Bunyavuridae	 Spherical	80–120	11–23	Single-stranded RNA	2.50	0.400	[142,145]
Aino[a]	Bunyavuridae	 Spherical	80–120	11–23	Single-stranded RNA	3.45	0.290	[142,146]

APV: avian poxvirus, PI3: parainfluenza virus type 3, IBR: infectious bovine rhinotracheitis, BVDV: bovine viral diarrhea virus, BEFV: bovine ephemeral fever virus.

$\log_{10}$ values for virus inactivation by γ-irradiation, as shown in Table 2 [135]. Multiple causative parameters may be involved in this discrepancy, including, but not limited to, sera matrices preparation variability from sample-to-sample, variability in γ-irradiation procedures (dose rate), and variability in the purity of the virus stock quality.

In another study, Schmidt and workers [132] investigated the fractionated and continuous electron beam irradiation of four different types of viruses: the Human Immunodeficiency Virus-2 (Retroviridae enveloped HIV-2), the Hepatovirus A (Picornaviridae non-enveloped HAV), the Pseudorabies Virus (Herpesviridae, enveloped PRV), and the Porcine Parvovirus (Parvoviridae non-enveloped PPV). The cell lines for these viruses were respectively human T lymphocyte cells, mink-lung cells, embryonal rhesus monkey kidney cells, and pig kidney cells. The irradiation doses for the continuous beam were multiple

of 3.4 kGy and up to 34 kGy, while for the fractionated beam, a dose of 3.4 Gy was applied up to 10 times. The D_{10} values were estimated from the regression curve $TCID_{50/mL}$ vs absorbed dose. Except for PRV, each type of virus showed a slightly greater radioresistance in continuous than fractionated irradiation. This might be explained by the ability of the viruses to repair their sublethal damages to their viral genome between two successive fractions. The least resistant was the PRV (continuous beam: 5.6 kGy, fractionated beam: 5.8 kGy) and the most resistant was the HIV-2 (continuous beam: 9 kGy, fractionated beam: 8 kGy).

The presence of solutes in the irradiation culture of viruses renders the radiation-induced inactivation of viruses less efficient. de Roda Husman et al. [125] compared the inactivation of the respiratory feline calicivirus (FeCV) and the enteric canine calicivirus (CaCV) with the Escherichia bacteriophage MS2 in viral culture of different protein concentration. They reported a $3\log_{10}$ reduction factor at a dose of ~120 Gy when γ-irradiating MS2 in tap water or in low-protein-contents. They obtained doses of 500 and 300 Gy, for a $3\log_{10}$ reductions factors, for FeCV and CaCV, respectively, in low-protein-content cultures. No or very little inactivation was observed when MS2, FeCV, and CaCV were γ-irradiated in high-protein-content culture. The presence of $OH^{\cdot}$ and $H^{\cdot}$ scavengers, such as proteins, lipids, etc., significantly reduces the viral inactivation. The authors also reported that FeCV and CaCV showed a second-order kinetics, with faster inactivation happening at doses between 0 and 400 Gy and slower inactivation happening at doses from 400 to 800 Gy.

2 Conclusion

The eruption of the SARS-CoV-2 new virus poses a real challenge and worries in a sense that its attributes are initially unknown and very limited viral data on COVID-19 infection is currently available. The virus is still infecting populations globally. Previous studies from other viruses and particularly the SARS-CoV and MERS-CoV have showed that damaging the genetic material can destroy infectivity while retaining immunogenicity. This review showed that ionizing radiation can potentially inactivate almost all the RNA viruses, by disrupting their genomic material. This could suggest a basis for developing a potential γ-inactivated virus-based vaccine against the spread of the COVID-19 pandemic. Improving its immunogenicity and preventing any potential undesired effects that could compromise the safety of the vaccine are of course other important factors which should also be taken into full consideration and thoroughly examined.

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