Iranian Journal of Virology 2019;13(1): 9-15
©2018, Iranian Society of Virology

Original Article

Comparison of Two Techniques, Gamma Irradiation and Acidic pH, on Biological Attributes of Interferon Beta-1a

Farahnaz Motamedi-Sedeh[*1], Sayed Mohammad Rasoulinejad-Mousavi[2], Masoomeh Sharbatdaran[3], Arash Arbabi[4]

1. Nuclear Agriculture Research School, Nuclear Science and Technology Research Institute, Moazen Street, Karaj, Iran
2. Department of Mirobiology and Microbial Biotechnology, Life Science and Biotechnology Faculty, Shahid Beheshti University
3. Nuclear Science and Technology Research Institute, Tehran, Iran
4. School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Abstract

Background and Aims: IFN beta-1a is a recombinant protein which reduces the symptoms of Multiple Sclerosisdisease. The purpose of this study was comparison the effects of gamma irradiation and acid pH methods on the biological attributes of recombinant protein IFN beta-1a in order to viral inactivation.

Materials and Methods: Methods: IFN beta was produced by recombinant-DNA in CHO Cell line which include human IFN beta gene and divided in two parts. The acidity of one part was adjusted about pH=4 by hydrochloric acid and incubated two hours, then was returned back to initial pH=7 by NaoH (1 M). The second part was irradiated by a Nordian model 220 gamma irradiator, with different doses: 10, 25 and 50 kGy. The prepared samples with both methods were analyzed by SDS-PAGE and HPLC.

Results: The HPLC indicated decreasing in purity and protein content of gamma irradiated samples, whereas these properties for the acidic pH treated sample was the same as the standard sample. Aggregation of gamma irradiated IFN beta-1a protein and increasing of the protein molecular weight were shown by SDS-PAGE, but it was without any change in molecular weight (22.5 KDa) at acidic pH sample. Also, decreasing of IFN beta antiviral activity through decreasing its concentration has been shown via antiviral assay.

Conclusions: Conclusion: The low pH method can be used for viral inactivation without any change in structure and biological activity of IFN beta-1a but the results indicated differences between the irradiated and standard samples, whereas the acidic pH treated sample was the same as the standard.

Keywords: Interferon Beta-1a, Gamma Irradiation, Low pH, Viral Inactivation, Biological Activity.

Introduction

Interferon beta-1a, which is used to reduce the symptom of Multiple Sclerosis disease,isa cytokine in he interferon family. It is a recombinant protein with 22.5 KD molecular weight and 166 amino acids. It normally produces by fibroblasts that have antiviral and anti-proliferative characteristics [1]. Also, it was produced by recombinant-DNA in CHO Cell line which include human IFN beta gene [1, 2]. It can balance the expression of pro- and anti-inflammatory agents in the brain, and reduces the number of inflammatory cells. Interferon beta can reduce neuron inflammation, increase production of nerve growth factor and improve neuronal survival [3, 4]. Viral safety is an important

[*] **Corresponding author:**
Farahnaz Motamedi-Sedeh, Moazen Street, Karaj, Iran, farah.motamedi@gmail.com; fmotamedi@aeoi.org.ir, Tel:+982634257075, Fax:+98263446061, orcid.org/ 0000-0003-3388-9670.

[Downloaded from journal.isv.org.ir on 2026-09-10]

characteristic of the biological products which derives from animal cell lines and use for therapeutic intent [5]. The risk of viral infection is one of the common problems for all cellular biological products such as IFN-beta 1a. So this contamination can have serious clinical consequences and its source can be from the cell line or due to infection with the virus during production [1]. MS is mainly characterized by demyelination of axonal tracks in the CNS which due to infiltration of perivascular CD4+ T-cell and mononuclear cells [6]. There are some mechanisms which IFN-beta causes anti-inflammatory and immune-modulatory effects, including inhibition of T-cell activation and proliferation; apoptosis of auto-reactive T cells; induction of regulatory T cells; prevention of leukocyte movement across the blood-brain barrier; cytokine modulation; and potential antiviral activity. These mechanisms are believed to be effective for therapeutic effect of IFN-beta in the treatment of Relapsing-Remitting Multiple sclerosis† [7]. With these interpretations, it is expected that the health of these products due to the possibility of viral contamination is ensured by using an antiviral assay program and evaluating the inactivation and elimination of the virus in the production process. The Low pH method is used to inactivate the viruses that make contamination and it is commonly used in monoclonal antibody purification processes to inactivate large enveloped viruses [8]. PH adjustment is very important in this method and is carried out by specialist personnel in special reservoirs [9]. Gamma irradiation is another technique for virus inactivation.

Ionizing radiation is a highly reliable procedure in which electromagnetic radiation such as gamma rays are emitted from Cobalt 60 or Cesium 137 isotopes used for inactivation of microorganisms such as viruses [10]. The inactivation of viruses by ionizing radiation shows that a single ionization is sufficient to inactivate a single virus particle [11].

Evidently, the inactivation dose is related to the virus concentration, the size of the viral particles and viral genomes, and the temperature of irradiation. Consequently, there is two mechanisms of virus inactivation by gamma irradiation: direct and indirect. Direct virus inactivation by gamma irradiation is mainly caused by radiolytic cleavage or crosslinking of genetic material. Also, direct damages on proteins and viral envelopes are caused by gamma irradiation. Indirect effects of gamma irradiation are caused by the action of radicals such as .OH, due to the radiolytic cleavage of water, and ozone which created from the radiolytic cleavage of O2. Viral nucleic acids and viral proteins can react with this molecules. Stop of viral nucleic acids replication, via both direct and indirect effects, is the main mechanism of virus inactivation by gamma irradiation [5, 12, 13, 14, 15]. The aim of this study is to compare the effects of gamma irradiation and acid pH methods on the biological attributes of recombinant interferon beta-1a protein in order to viral inactivation.

Methods

Interferon beta 1a Production. It was produced by rDNA in CHO Cell‡ line which include human IFN beta gene. The supernatant of CHO Cells was collected, filtrated by 0.22 µm filter and purified by ion exchange chromatography [16].

Low pH Method. The reservoir temperature was equilibrated to 2-8 ˚C and the purified interferon beta-1a sample was incubated in this reservoir at pH= 4 with hydrochloric acid for 2 hours, then readjusted to the initial pH of 6.8 to 7.2 [16, 17, 18].

Gamma Irradiation Technique. Nine vials contain IFN beta-1a were irradiated by a Nordian model 220 gamma cell instrument at a dose rate of 2.07 Gy/s and activity of 8677 Ci at Nuclear Science and Technology Research Institute of Tehran with different doses 10, 25 and 50 kGy.

Three samples were irradiated for each dose of gamma ray at the freezing tem-perature and returned to the laboratory under freezing conditions[19].

[Downloaded from journal.isv.org.ir on 2026-09-10]

Table 1. Gamma Irradiated, Standard, Low pH samples results for RP-HPLC and SE-HPLC to calculate protein content (measured by Agilant 1260 HPLC devices)

Sample name	test	Retention time (min.)(Main Peak)	Area (mAU)	Area%
(A) Standard samples of IFN β-1a	SE-HPLC (16-23 min.)	18.779	1615.401	94.124
(B) 50 kGy Irradiated IFN β-1a	SE-HPLC (16-23 min.)	19.682	19.447	2.393
(C) 25 kGy Irradiated IFN β-1a	SE-HPLC (16-23 min.)	17.865	341.396	25.413
(D) 10 kGy Irradiated IFN β-1a	SE-HPLC (16-23 min.)	18.502	656.763	44.609
(E) Low pH treated IFN β-1a	SE-HPLC (16-23 min.)	18.372	1615.207	94.120
(A) Standard samples of IFN β-1a	RP-HPLC (22-26 min.)	23.363	22009.287	100
(B) 50 kGy Irradiated IFN β-1a	RP-HPLC (22-26 min.)	0.000 Less than LOD	0.000 Less than LOD	0.000 Less than LOD
(C) 25 kGy Irradiated IFN β-1a	RP-HPLC (22-26 min.)	0.000 Less than LOD	0.000 Less than LOD	0.000 Less than LOD
(D) 10 kGy Irradiated IFN β-1a	RP-HPLC (22-26 min.)	0.000 Less than LOD	0.000 Less than LOD	0.000 Less than LOD
(E) Low pH treated IFN β-1a	RP-HPLC (22-26 min.)	23.367	22008.889	100

Biological Activity. The protein structure and biological activity of interferon beta 1a were evaluated after treatment with low pH and gamma irradiation. Anti-viral assay and Sodium Dodecyl Sulfonate–Polyacrylamide Gel Electrophoresis technique were used for considering biological activity and protein structure; SDS-PAGE on a 12.5% Tris-HCl gel concentration under reducing conditions was done. The gel was stained with Coomassie-R250 stain (Merck). The control reference standard with concentration 1.5 mg/ml was prepared by spectrophotometer Nano-Drop and electrophoresis executed according to European pharmacopeia version 18, section 1639 [16]. Anti-viral assay of IFN beta-1a was performed on A549 cell line by preventing cytopathic effects of Encephalomyocarditis virus (EMC virus) (Picornaviridae family).

A serial dilution of IFN beta-1a (two-fold dilution from 16- 0.125 IU/ml) and 10 PFU/ml of EMC virus were mixed then inoculated on A549 cell line. Each of treated samples (standard sample and acidic pH treated sample) was examined in three repetitions. Optical Density§ was read by plate reader at 610 nm and biological activity calculated by GEN5 software.

Quality Control to Evaluate of IFN beta-1a Purification and Concentration. Size Exclusion High Performance Liquid Chromatography can separated proteins based on size and molecular weight. This technique was used to show the purification of IFN beta-1a by Agilent model 1260 SE-HPLC system, TOSOH resin with dimensions of 60 cm*7.8mm and detector with 280 nm. The runtime was 60 minutes and 100 µl of the sample (30µg/ml) was injected at runtime 60 minutes. Phosphate buffered as the mobile phase and a standard sample (0.2 mg/ml) were used [17]. Revers Phase High Performance Liquid Chromatography was used to determine concentration of IFN beta-1a. For RP-HPLC, a C4 column of 4.6mm x 150mm was used. The injection rate was 50 µl/min and the runtime was 40 minutes, using a wavelength of 214 nm. Water with trifluoroacetate and water along with TFA and acetonitrile were used for injection as the mobile phase A and B, respectively.

In this method a standard sample 100 µg/ml was used [17].

[Downloaded from journal.isv.org.ir on 2026-09-10]

Results

The results of SDS-PAGE for standard IFN beta-1a, acidic pH and gamma irradiated samples were shown in Figure 1 with different

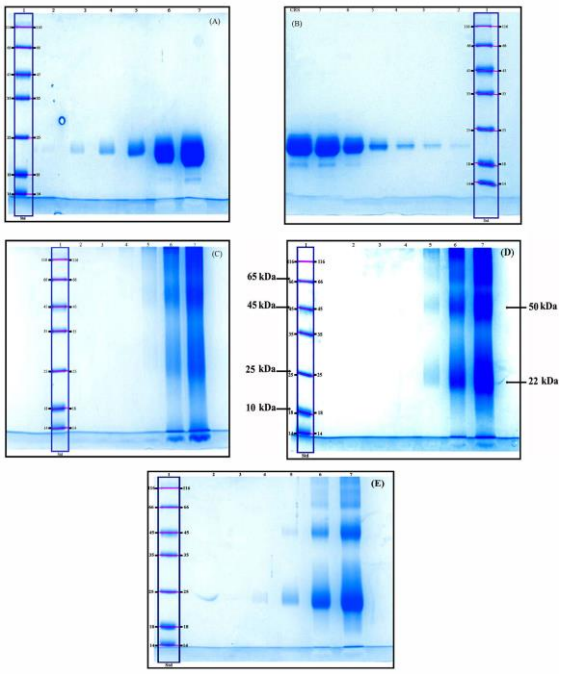

Fig. 1. The results of SDS-PAGE: (A) Standard samples of IFN-β 1a, (B) Acidic pH treated IFN-β 1a, (C) Gamma irradiated IFN-β 1a (50 kGy), (D) Gamma irradiated IFN-β 1a (25 kGy), (E) Gamma irradiated IFN-β 1a (10 kGy). Column 1; Unstained Protein Marker 26610, columns 2-7; Standard samples of IFN-β 1a with 0.00375 mg/ml, 0.0075 mg/ml, 0.015 mg/ml, 0.05 mg/ml, 0.3 mg/ml and 0.75 mg/ml concentrations.

concentration. A protein band with 22 kDa molecular weight was observed for all dilutions of standard IFN beta-1a on the gel. Also, the molecular weight of different concentration for acidic pH treated samples was the same. The columns four, five, six, and seven of gamma irradiated IFN beta-1a have inaccurate bands, in addition to a band of about 22 kDa, which is the same as interferon beta. There were the bands in the range of 50 kDa, which indicating the aggregation of protein molecules by radiation.

The result of RP- HPLC to check purity of all samples and SE-HPLC to calculate protein content were shown in Figures 2 and 3. These results showed that gamma irradiation

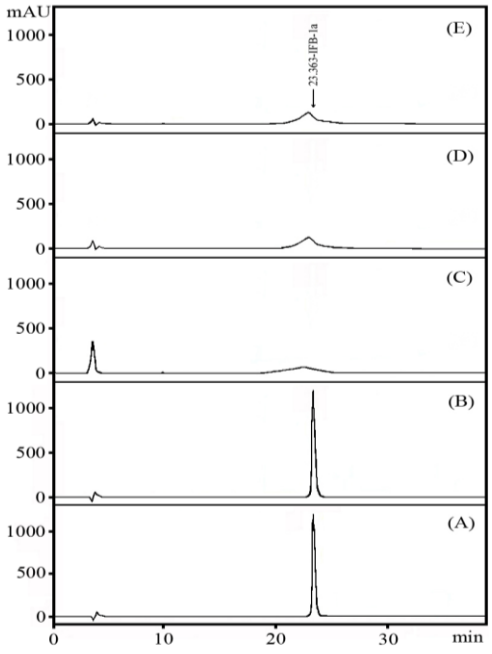

Fig. 2. The results of RP-HPLC: (A) Standard samples of IFN β-1a, (B) Low pH treated IFN β-1a, (C) 50 kGy Irradiated IFN β-1a, (D) 25 kGy Irradiated IFN β-1a, (E) 10 kGy Irradiated IFN β-1a.

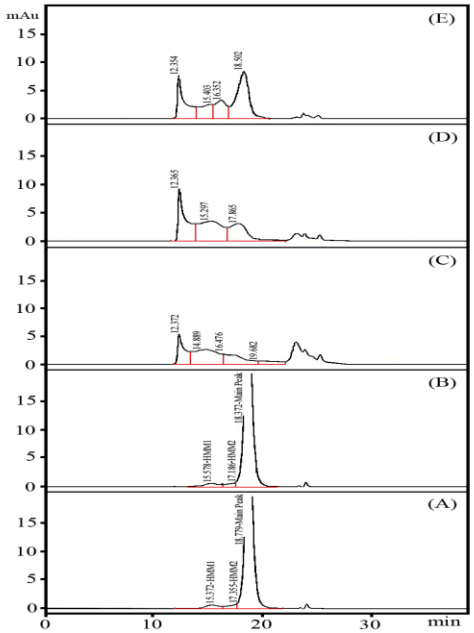

Fig. 3. The results of SE-HPLC: (A) Standard samples of IFN β-1a, (B) low pH treated IFN β-1a (C) 50 kGy Irradiated IFN β-1a, (D) 25 kGy Irradiated IFN β-1a, (E) 10 kGy Irradiated IFN β-1a.

damaged on IFN beta-1a, but the purity of acidic pH treated IFN beta-1a was the same of standard sample purity. Analysis method was

[Downloaded from journal.isv.org.ir on 2026-09-10]

RP-HPLC to calculate protein content after different treatments in comparison with standard sample without any process. RP-HPLC showed a peak of IFN β-1a standard sample at 20 minutes, but for gamma irradiated samples it decreased, so the amount of IFN beta-1a became less.

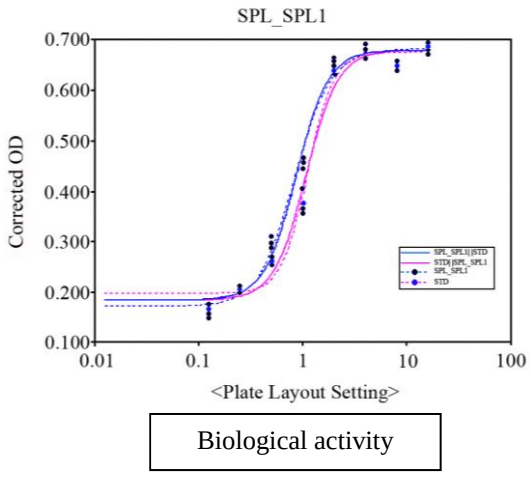

Fig. 4. Compatibility diagram for Low pH sample and standard by GEN 5 software.

SPL_SPL1‖STD:Sample No.1(Low pH Sample) paralel with Standard Sampl

STD‖SPL_SPL1:Standard Sample Paralel with Sample No.1(Low pH Sample)

Triplicate point of Sample No.1(Low pH Sample)

Triplicate points of Standard Sample

As mention in Table 1 there are significant differences between gamma irradiated and standard samples for RP and SE-HPLC in retention time, area of main peak and area percent.

However, low pH sample and standard IFN beta-1a were similar and there is no significant difference between them for RP-HPLC and SE-HPLC in retention time, area of main peak and area percent. So, low pH method can be used for viral inactivation without makes any change in protein recombinant characteristics. The result of anti-viral assay as the biological activity of low pH treated sample and standard sample were shown in Figure 4. Anti-viral activity of IFN beta-1a after treatment with acidic pH did not change and the same with standard sample. As mentioned in GEN 5 Graph, triplicate points of each sample in different dilutions clear that there is no significant difference between OD of standard and low pH samples.

Discussion

Gamma radiation and low pH techniques are the most common methods for viral inactivation that are carried out for products containing protein. In this study, both methods are used to choose a more effective method while protecting the biological properties of the target protein. Hume et al., studied about inactivation of some RNA viruses by gamma-ray. They evaluated gamma radiation effects on Zaire Ebola Virus in culture media and biological samples. They reported the viral neutralization in ambient conditions at biosafety level 4 (BSL -4) [4]. Lee et al., reviewed chemical and gamma-ray methods in order to neutralizef enveloped and non-enveloped viruses in products such as serum. Based on this study, treatment with 70% ethanol and 4% sodium hydroxide and gamma radiation has been highly effective in virus neutralization [20]. Erwin Duzer et al. (2004) studied the inactivation of enteric canine calicivirus and the respiratory feline calicivirus and they reported which inactivation is relevant to reduction in PCR units of FeCV, CaCV, and a norovirus. Virus inactivation was temperature and time dependent, also UV-B radiation caused dose-dependent inactivation, with a 3D (D= 1 log10) reduction in infectivity at 34 mj/cm2 for both viruses. Inactivation by 70% ethanol was inefficient, with only 3D reduction after 30 min. Sodium hypochlorite solutions were only effective at >300 ppm. Both animal viruses showed similar inactivation profiles when exposed to heat or UV-B radiation or when incubated in ethanol or hypochlorite [21, 22]. Irradiated inactivated viruses have been reported to retain most of their antigenicity. Motamedi et al. (2008) showed that the optimum dose range of gamma ray for inactivation of FMD Virus typeA87/ IRN at -20 ˚C, without any change in antigenicity was obtained 40-44 kGy. Therefore, the irradiated inactivated FMD virus with unaltered antigenicity character and good safety test results can be used to prepare

[Downloaded from journal.isv.org.ir on 2026-09-10]

of inactivated irradiated vaccine [23, 24].
Motamedi et al. (2017) reported that White Spot Syndrome Virus was inactivated by the electron beam irradiation, D10 value and optimum dose of electron beam were obtained at 1.85 and 13 kGy, respectively [23]. Also, Motamedi et al. (2015) in another research reported that comparison of the immune responses between gamma ray-irradiated vaccine and conventional vaccine against FMD virus did not show any significant difference in memory spleen T lymphocytes, neutralizing antibody titer or IFN-γ, IL-10, IL-4 and IL-2 concentrations ($p > 0.05$). In contrast, there were significant differences in all of the evaluated immune factors between the two vaccinated groups of mice and negative control mice ($p < 0.05$) [10]. According to the above results we can suppose this concept, rather than viral inactivation is done after gamma radiation but the viral proteins structure in capsid as a whole viral particle don't change. Low pH inactivation is commonly used to inactivate enveloped viruses in purified monoclonal antibody. Acidic low pH has been confirmed as a reliable method to inactivate more than >4 log10 of large enveloped viruses (e.g., X-MuLV) in several commercial purification processes. This method can be applied for monoclonal antibodies which the purification process steps include a low pH step [3]. In this study two methods were used for viral inactivation of IFN beta-1a which infected by viral contamination (e.g., EMC virus) and the results of the HPLC and SDS-PAGE indicated that there was difference between the irradiated sample and standard sample, but the acidic pH sample was the same of the standard sample. The SDS-PAGE were shown IFN beta-1a protein (monomer with molecular weight 22 kDa) was aggregated after gamma irradiation, so the protein band with increased molecular weight was observed (about 50 kDa), but it was without any change in molecular weight (22.5 KDa) at acidic pH sample. Whereas the monomer molecules of IFN beta-1a have the small size, so there is less biomass for irradiation and it causes more sensitivity to gamma radiation. Whilst in some articles reported more resistance for other viral proteins such as FMD virus (Picornaviridea family) which is resistance against 44 kGy gamma radiation, because viral proteins are attached with each other with tight connection in capsid structure, but the IFN beta-1a molecules are monomer protein with small biomass [11, 23, 25, 26]. Also, the result of antiviral assay was shown decreasing of IFN beta antiviral activity according to decreasing IFN beta-1a concentration in acidic treated sample and standard sample. Eventually the low pH method can be used for viral inactivation without any change in structure and biological activity of IFN beta-1a. Although gamma irradiation can inactivate viruses but it makes aggregation for this small peptide, so it is not a suitable technique for viral inactivation effects on the small peptide such as IFN beta-1a.

Acknowledgment

The authors would like to express their gratitude to Cinnagen Company in Karaj, Iran for their support and Nuclear Science and Technology Research Institute of Iran.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Murdoch D, Lyseng-Williamson KA. Spotlight on subcutaneous recombinant interferon-beta-1a in relapsing-remitting multiple sclerosis". BioDrugs 2005;19(5): 323-5.
2. Giovannoni G, Munschauer FE, Deisenhammer F. Neutralizing antibodies to interferon beta during the treatment of multiple sclerosis. J Neurol Neurosurg Psychiatry. 2002;73(5):465–9.
3. Kieseier BC. The mechanism of action of interferon-β in relapsing multiple sclerosis. CNS Drugs 2011;25 (6):491-502.

[Downloaded from journal.isv.org.ir on 2026-09-10]

4. Markowitz CE. Interferon-beta: mechanism of action and dosing issues. Neurol. 2007;68(24: 4): S8-11.
5. Hume AJ, Ames J, Rennick LJ, Duprex WP, Marzi A, Tonkiss J, et al. Inactivation of RNA Viruses by Gamma Irradiation. Virus 2016; 8: 204- 212.
6. Soleimani M , Jameie SB, Barati M, Mehdizadeh M, Kerdari M. Effects of Coenzyme Q10 on the ratio of TH1/TH2 in Experimental Autoimmune Encephalomyelitis Model of Multiple Sclerosis in C57BL/6. Iran Biomed J. 2014;18(4):203-211.
7. Dhib-Jalbut S, Marks S. Interferon-β mechanisms of action in multiple sclerosis. Neurol. 2010;74(1): S17-24.
8. Makowiecki J, Mallory H. Adjusting pH During Viral Inactivation. Toturi 2013; 33(8). https://www.genengnews.com/gen-articles/adjusting-ph-during-viral-inactivation/4838.
9. Mattila J, Clark M, Liu S, Pieracci J, Gervais TR. Retrospective Evaluation of Low-pH Viral Inactivation and Viral Filtration Data from a Multiple Company Collaboration. PDA. J Pharm Sci Technol. 2016;70: 293 -9.
10. Motamedi-Sedeh F, Afsharnasab M, Heidarieh M. Immunization of Litopenaeus vannamei shrimp against white spot syndrome virus (WSSV) by gamma-irradiated WSSV plus Vibrio paraheomolyticus. Vaccine Res. 2015;2(5):107-111.
11. Motamedi-Sedeh F, Soleimanjahi H, Jalilian AR, Mahravani H, Shafaee K, Sotoodeh M. Development of Protective Immunity against Inactivated Iranian Isolate of Foot-and-Mouth Disease Virus Type O/IRN/2007 Using Gamma Ray-Irradiated Vaccine on BALB/c Mice and Guinea Pigs. Intervirol. 2015;58(3):190-6.
12. Wang W, Yu Z, Su W. Ion Irradiation and Biomolecular Radiation Damage II. Indirect Effect. 2010, arXiv: 1004.4394 [physics.bio-ph].
13. Lomax M.E, Folkes L.K, O'Neill P. Biological consequences of radiation-induced DNA damage: Relevance to radiotherapy. Clin Oncol. 2013;25:578–585.
14. Ohshima H, Iida Y, Matsuda A, Kuwabara M. Damage induced by hydroxyl radicals generated in the hydration layer of gamma-irradiated frozen aqueous solution of DNA. J Radiat Res. 1996;37:199–207.
15. Summers W.C.; Szybalski W. Gamma-irradiation of deoxyribonucleic acid in dilute solutions. II. Molecular mechanisms responsible for inactivation of phage, its transfecting DNA, and of bacterial transforming activity. J Mol Biol. 1967;26:227–235.
16. European Pharmacopeia. Interferon beta-1a concentrated solution. 2008;1639e (6.3): 4177-80.
17. Dias PVS, Arthuso FS, Oliveira JE, Suzuki MF, Sousa JM, Ribela MTCP, et al. Determination of recombinant Interferon-α2 in E. coli periplasmic extracts by reversed-phase high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci. 2018;1(1072):193–198.
18. Utsumi J, Yamazaki S, Kawaguch K, Kimura S, Shimizu H. Stability of human interferon-ß1: oligomeric human interferon-ß1 is inactive but is reactivated by monomerization. Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology. 1989; 998(2):167-172.
19. International Atomic Energy Agency (1990) Guidelines for Industrial Radiation Sterilization of Disposable Medical Products (Cobalt-60 Gamma Irradiation), in Technical Reports Series. International Atomic Energy Agency: Vienna 539: 39.
20. Lee KI, Lee JS, Jung HH, Lee HY, Moon SH, Kang KT, et al. Inactivation of enveloped and non-enveloped viruses in the process of chemical treatment and gamma irradiation of bovine-derived grafting materials. Xenotransplantation. 2012;19(6): 365-9.
21. Duizer E, Bijkerk P, Rockx B, Groot A, Twisk F, Koopmans M. Inactivation of Caliciviruses. Appl Environ Microbiol. 2004;70(8):4538–43.
22. Husman AM, Bijkerk P, Lodder W, Berg HV, Pribil W, Cabaj A, et al. Calicivirus Inactivation by Nonionizing and Ionizing (Gamma) Radiation. Appl Environ Microbiol. 2004; 70(9): 5089–5093.
23. Motamedi Sedeh F, Khorasani A, Shafaee SK, Fatolahi H, Arbabi K. Preparation of FMD type A87/IRN inactivated Vaccine by gamma irradiation and the immune response on guinea pig. Indian J Microbiol. 2008;48(3):51-60.
24. Motamedi-Sedeh F, Afsharnasab M, Heidarieh M, Tahami SM. Protection of Litopenaeus vannamei against white spot syndrome virus by electron-irradiated inactivated vaccine and prebiotic immunogen. Radiat Phys Chem. 2017;130:421–5.
25. Lombardo J H, Smolko E. A Biotechnology project with agamma radiation source of 100,000 Ci. Radiat Phys chem. 1990; 35 (4-6): 585-589.
26. Smolko E E, Lombardo J H. Virus inactivation studies using ion beams, electron and gamma irradiation. Nucl Instru Methods Phys Res B. 2005;236:249-53.

[Downloaded from journal.isv.org.ir on 2026-09-10]