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Preparation and evaluation of a [^{66}Ga]gallium chitosan complex in fibrosarcoma bearing animal models

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Abstract. [^{66}Ga]gallium chitosan complex was prepared with a high radiochemical purity (> 99%) in dilute acetic acid solution. The radiochemical purity of [^{66}Ga]gallium chitosan complex was checked by using paper chromatography technique. The prepared complex solution was injected intratumoral to fibrosarcoma-bearing mice and the leakage of radioactivity from injection site was investigated. Approximately, 85.4% of the injected dose was retained in the injection site 54 h after injection and most of the leaked radioactivity was accumulated in the blood, liver (0.5%) and lung (6.5%).

Key words: chitosan • gallium-66 • internal radiotherapy • fibrosarcoma • intratumoral injection

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Introduction

Intratumoral injection of therapeutic radionuclides is an attractive approach to restrict radiation delivery to a defined tumor area. In this approach, a non-removable form of therapeutic radionuclide is injected directly into a tumor lesion to minimize the radiation side effects on normal tissues. Intratumoral injection of several non-removable radiolabeled agents such as holmium-165 loaded poly-L-lactide acid (PLLA) nanoparticles [7], ^{90}Y particles embedded in an alginate gel [8], ^{90}Y glass microspheres [3, 17], ^{32}P glass microspheres suspended in gelatin [34], rhenium-188 sulfide suspension [13, 14] has already been reported and they have shown a suitable efficiency in preventing the leakage of radioactivity from injection site.

Chitosan, a natural and biodegradable polysaccharide [16, 26], is an excellent molecule for preparing non-removable radiolabeled agents. Chitosan produces stable complexes with radioactive metal ions depending on metal, pH, and matrix of the solution [6, 19]. Since aqueous chitosan solutions are viscose, stability of the prepared complex and viscosity of solution could prevent the leakage of radioactivity from injection site after intratumoral injection. Ho-166 chitosan [21–23, 30] and Sm-153 chitosan [29] complexes have been reported for internal radiation therapy and treatment of such diseases as hepatocellular carcinoma and rheumatoid arthritis. However, the efficiency of chitosan for

complexing other radioisotopes as well as its ability for preventing the leakage of radioactivity in the treatment of various tumors could be considered.

Gallium-66, a carrier free cyclotron produced radioisotope ($T_{1/2}$ = 9.49 h, β^+ abundance 56.5%, E_{mean} = 1.7 MeV, mean range in tissue 28 mm) decays to Zn-66 by the most abundant positron β^+ (51.5%) and E_{max} = 4.2 MeV [5]. Positrons, while transferring through materials, act approximately like electrons losing their energy before complete annihilation. Positrons emitted from gallium-66 can damage deoxyribonucleic acid (DNA) of cancerous cells without being entered gallium-66 into the cells [9, 18, 20, 24, 25, 28]. Therefore, gallium-66 could be considered as a radiotherapy agent [15, 32].

In this research, a gallium-66 chitosan complex was prepared and the effect of several parameters such as molecular weight and concentration of chitosan, pH of chitosan solutions, gallium-66 radioactivity and time was investigated on its radiolabeling yield. Gallium-66 chitosan complex solution was injected intratumoral to fibrosarcoma-bearing mice and the leakage of radioactivity from injection site was investigated. Primary results of this research have been reported at the 17th International Symposium on Radiopharmaceutical Science [1].

Experimental

Materials and instruments

Two kinds of chitosan (medium molecular weight, MW = 400 kDa, degree of deactylation (DDA) = 85% and high molecular weight, MW = 600 kDa, DDA = 85%) were obtained from Fluka (Bucks, Switzerland). Chromatography paper, Whatman no. 1 was obtained from Whatman (Maidstone, UK). Radiochromatography was performed by using a Bioscan AR-2000 radio thin-layer chromatography (TLC) scanner instrument (Washington DC, USA). A radioactive dose calibrator ISOMED 1010 (Dresden, Germany) and a high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer were used for counting distributed radioactivity in mice organs. AG 50W, 200–400 mesh, H^+ form cation exchange resin was obtained from BioRad Labs (Richmond, California, USA). All other chemical reagents were purchased from Merck (Darmstadt, Germany).

Preparation of [^{66}Ga]gallium chloride solution

[^{66}Ga]gallium chloride was prepared by 15 MeV proton bombardment of an electroplated enriched Zn-66 target in a 30 MeV cyclotron (IBA-Cyclone 30) and purified by a no-carrier-added method described previously [27, 36]. Briefly, the irradiated target was dissolved in 10 M HCl (15 ml). The obtained solution was passed through a cation exchange resin (BioRad AG 50W, 200–400 mesh, H^+ form) which had been preconditioned by passing 25 ml of 9 M HCl. The column was then washed with 25 ml of 9 M HCl to remove copper and zinc ion contents. Consequently, Ga-66 cation was washed out by 20 ml of 4 M HCl. The resultant solution was dried under a stream of nitrogen gas at 60°C. The residue was dissolved in 2 ml of deionized water and dried for a second time. Eventually, the residue was dissolved in 5 ml of deionized water. The final [^{66}Ga]gallium chloride solution (300 mCi/ml) was examined for chemical purity by differential-pulsed anodic stripping polarography and for radionuclide purity using a HPGe spectrometer [27]. Radionuclide purity was higher than 97% and the amounts of chemical impurities were in accordance with the United States Pharmacopoeia (USP) levels.

Synthesis of [^{66}Ga]gallium chitosan complex

To prepare [^{66}Ga]gallium chitosan complex, chitosan was dissolved in 3 ml of 0.16 M aqueous acetic acid solution and then [^{66}Ga]gallium chloride solution was added. The obtained solution was stirred for 5 min and then was kept for 30 min at room temperature. To investigate the effect of several factors such as molecular weight of chitosan and its concentration on the labeling yield, two kinds of chitosan with molecular weights equal to 400 kDa and 600 kDa and several concentrations of medium molecular weight chitosan (MW = 400 kDa) varied in the range of 0.85–15.7 mg/ml were used. To investigate the effect of pH on the labeling yield, a series of chitosan solution were prepared by dissolving 30 mg samples of chitosan (MW = 400 kDa) in a series of 2 ml aqueous acetic acid solutions. The acidity of the resultant solutions were adjusted to desired pH which varied in the range of 1–6.5, by using 0.5 M NaOH and 0.5 M HCl. Then, the volume was adjusted to 3 ml and 30 mCi of [^{66}Ga]gallium chloride was added. On the other hand, to investigate the effect of time on the labeling yield, radiochemical purity was checked with the time up to 45 min for 11.6 mg/ml chitosan (MW = 400 kDa) solutions. Finally, the capacity of chitosan was examined by adding different [^{66}Ga]gallium chloride radioactivities which varied in the range of 30–120 mCi to a series of 11.6 mg/ml chitosan solutions.

Stability tests

Stability of the prepared [^{66}Ga]gallium chitosan complex was examined by measuring radiochemical purity using paper chromatography at 1, 2, 4, 8, 16, 24, 36 and 48 h after preparation. To investigate *in-vitro* the stability of [^{66}Ga]gallium chitosan complex, 200 µl of the complex solution was dispersed into 500 µl of 50 mM phosphate buffer solution (PBS), pH = 7. The mixture was incubated at 37°C for 2 days and radiochemical purity was checked by paper chromatography during incubation.

Quality control

To measure radiochemical purity and radiolabeling yield, a 1 µL sample of the [^{66}Ga]gallium chitosan complex was spotted on a chromatography paper (Whatman no. 1), and developed in a mixture of methanol/water/acetic acid (4.5:4.5:1) as the mobile phase. The R_f values of free Ga-66 and [^{66}Ga]gallium chitosan complex were 0.9 and 0.0–0.1, respectively.

Induction of fibrosarcoma tumors in mice

Tumor induction was performed by the use of polyaromatic hydrocarbon injection in rodents as reported previously [4, 11, 12]. To prepare a tumor model, 10 µl of 3-methyl cholanthrene solution in extra virgin olive oil (4 mg/ml) was subcutaneously (SC) injected to the dorsal area of the mice. After 14–16 weeks, the tumors weighed 0.2–0.4 g and were not grossly necrotic. Tumor tissues of some random animals were sent for pathological tests and were diagnosed as fibrosarcoma.

Animal studies

In order to perform *in vivo* studies, 100 µL (600 µCi) samples of [66Ga]gallium chitosan (MW = 400 kDa, 11.6 mg/ml) solution were injected directly to tumoral lesions of mice. Moreover, 100 µL (600 µCi) samples of [66Ga]gallium chloride solution were injected directly to tumoral lesions of another group of mice for the control study. To investigate the leakage of radioactivity, the animals (n = 3) were sacrificed by ether asphyxiation at selected times after injection (3, 9, 18, 27 and 54 h) and percentage of the injected dose in the tissues (brain, heart, liver, kidney, testis, spleen, lung, stomach, bladder, carcass and tumor) were determined either by a method of γ-ray scintillation or a dose calibrator.

Result and discussion

Effect of various factors on the labeling yield

Figure 1a shows the effect of molecular weight and concentration of chitosan on labeling yield at pH = 3.5. Although the chitosan with a molecular weight of 600 kDa had a better efficiency for complexing radiogallium ions, but the resultant radiolabeled chitosan solution was highly viscose and its injection was very difficult. Thus, the chitosan with a molecular weight of 400 kDa was used for further experiments. Labeling yield increased with increasing chitosan (400 kDa) concentration reaching above 99% at the concentration of 11.6 mg/ml. The highest labeling yield achieved at pH = 3.1–3.9, while the yield decreased out of this range (Fig. 1b), which is due to this fact that the fully hydrated Ga^{3+} is only stable under acidic conditions. Once the pH has raised above 3, gallium begins to form anionic $[Ga(OAc)_n]^{3-n}$ in aqueous acetic acid solution [31, 35]. The protonated amine groups of chitosan in acidic pH are able to absorb metal anions. When the pH rises further, chitosan intends to be neutral and cannot absorb metal anions reducing radiolabeling yield [6]. The labeling yield of 99% was achieved after 30 min (Fig. 1c). The radiolabeling yield decreased slightly (2.5%), after the addition 4 times of radiogallium radioactivity to the chitosan solution (11.6 mg/ml).

Based on the results, the best procedure for the preparation of [66Ga]gallium chitosan complex with a high labeling yield is as follows. 35 mg of chitosan (MW = 400 kDa) was dissolved in 2 ml of 1% acetic acid aqueous solution. The acidity of the obtained solution was adjusted to pH = 3.5 by addition of 0.5 M NaOH solution and followed by addition of [66Ga]gallium chloride solution. Finally, the total volume was adjusted to 3 ml by addition of deionized water followed by stirring for 5 min and standing for 30 min at room temperature.

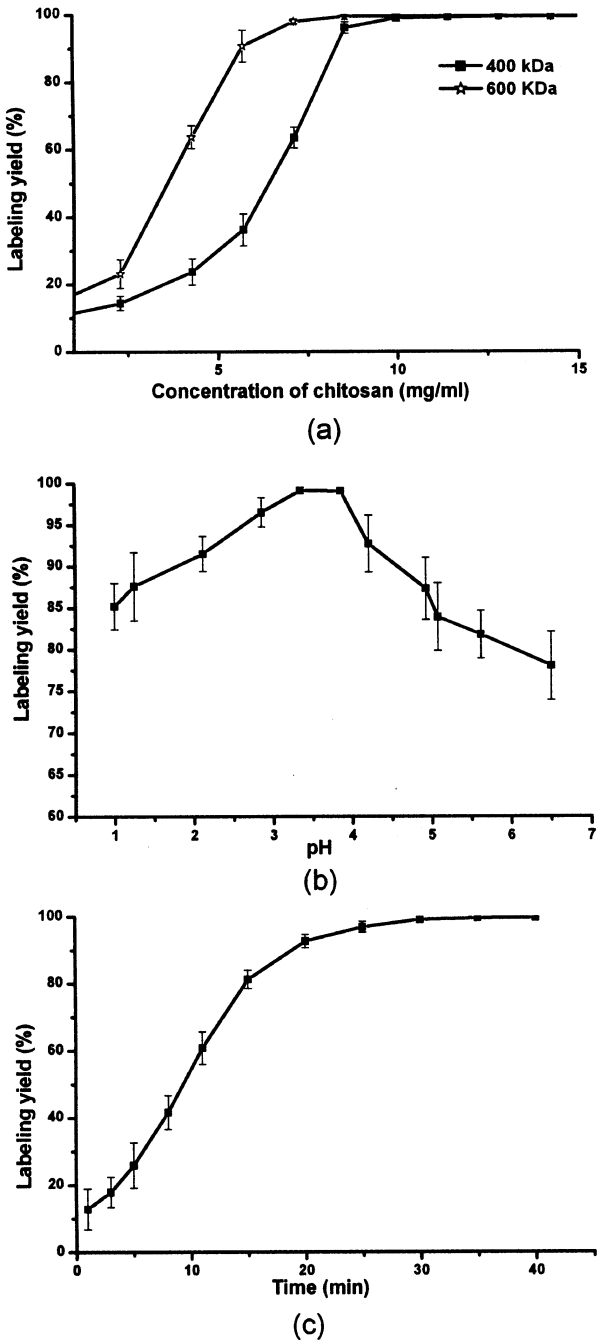

Fig. 1. Influence of several parameters on the labeling yield of [66Ga]gallium chitosan. a – molecular weight and concentration of chitosan; b – pH of chitosan solutions; c – time. Each point presents mean ± SD (n = 3).

Stability of [66Ga]gallium chitosan complex

The stability of the prepared [66Ga]gallium chitosan complex was checked with time up to 48 h after preparation (Fig. 2). The complex was stable in acidic solution (pH = 3.5) with radiochemical purity of 98% even 48 h after preparation. The radiochemical purity of [66Ga]gallium chitosan complex decreased to 93% after

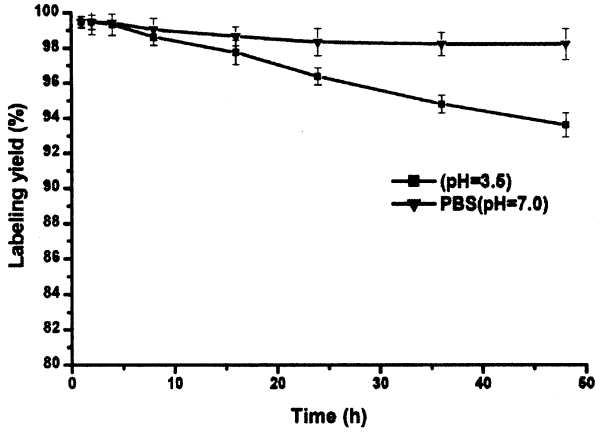

Fig. 2. Stability of [^{66}Ga]gallium chitosan. Each point presents mean ± SD (n = 3).

48 h in phosphate buffer solution, which indicates the [^{66}Ga]gallium chitosan complex is less stable than previously reported Sm-153 and Ho-166 chitosan complexes [22, 29, 30]. The decrease in radiochemical purity might be the reason of radioactivity leakage from injection site. However, the complex has been somewhat stable which prevents the leakage of major part of injected radioactivity even 54 h after injection.

Distribution of radioactivity

Figure 3 shows the distribution of injected dose in the mice organs for intratumoral injection of 600 µCi/100ml of [^{66}Ga]gallium chloride solution after 3 and 24 h, as percentage of the injected dose. Based on these results, it was concluded that the major portion of injected radioactivity was extracted to blood circulation and distributed in mice organs.

Figure 4 presents the distribution of injected dose in the mice organs after 3, 9, 18, 27 and 57 h of intratumoral injection of 600 µCi/100 µl of [^{66}Ga]gallium chitosan complex as percentage of the injected dose. Figure 4 shows that only a small portion of radioactivity has been released from the injection site to the blood circulation at first hours (up to 9 h). However, in the next hours, the radioactivity is diffused into blood circulation probably due to complex dissociation caused by chitosan biodegradation and/or pH change. The retention of injected radioactivity in injection site was 85.4% and the most of leaked radioactivity were in the blood, liver (0.5%) and lung (6.5%) 54 h after injection. The observed radioactivity retention for fibrosarcoma tumor model after intratumoral injection of Ga-66 chitosan complex is less than the previously reported radioactivity retentions for intrahepatic injection of Ho-166 chitosan complex (97% for 72 h) [30] and for intra-articular injection of Ho-166 and Sm-153 chitosan complexes (99% for 144 h) [23, 29]. This might be the result of lower stability of Ga-66 chitosan complex in biological pH as well as the difference between the properties of injection sites.

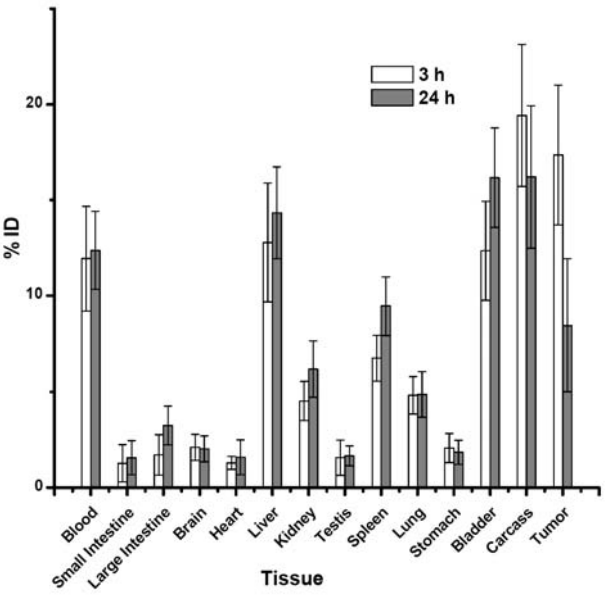

Fig. 3. Distribution of radioactivity in the fibrosarcoma-bearing mice organs, 3 and 24 h after intratumoral injection of 600 µCi of [^{66}Ga]gallium chloride solution. ID% – percentage of injected dose. Each bar presents mean ± SD (n = 3).

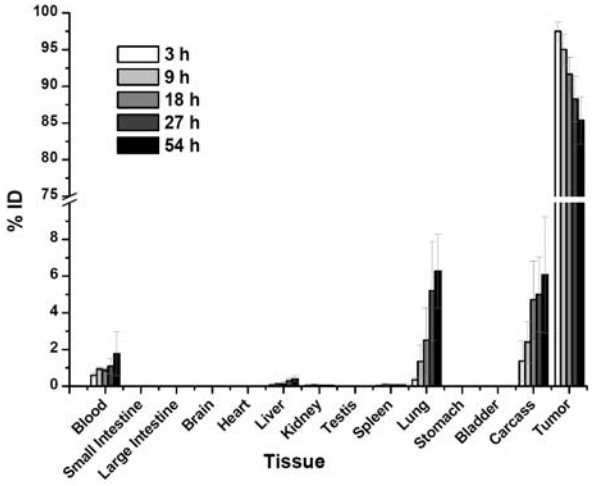

Fig. 4. Distribution of radioactivity in the fibrosarcoma-bearing mice organs, 3, 9, 18, 27 and 54 h after intratumoral injection of 600 µCi of [^{66}Ga]gallium chitosan solution. ID% – percentage of injected dose. Each bar presents mean ± SD (n = 3).

Conclusion

[^{66}Ga]gallium chitosan complex was prepared with a high radiochemical yield (> 99%) in the optimized conditions; 11.6 mg/ml of chitosan concentration in dilute acetic acid solution (pH = 3.5). The radiolabeled complex was stable in the preparation medium and can be used up to 24 h after preparation. The radiochemical purity of the radiolabeled complex decreased to 93% after 48 h in the 50 mM PBS (pH = 7). Intratumoral injection of [^{66}Ga]gallium chitosan complex to fibrosarcoma-bearing mice and investigation of leakage of radioactivity in the body showed that the retention of injected radioactivity in injection site was 85.4% and the most of leaked radioactivity were in the blood, liver (0.5%) and lung (6.5%) 54 h after injection. It seems that the acidity of tissue affects the acidity of injected solution and leads to a decrease of complex stability and releases the radioactivity into blood circulation. However, the leakage of radioactivity will be probably

decreased by using chemically modified chitosans which have more ability to form stable complexes in biological pH in comparison with chitosan [2, 10, 33, 35].

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References

1. Akhlaghi M, Rowshanfarzad P, Jalilian AR, Moradkhani S, Saddadi F (2007) Preparation of ^{66}Ga-chitosan for the endoradiotherapy (abstract). J Labelled Compd Rad 50;S1:S450
2. Chassary P, Vincent T, Guibal E (2004) Metal anion sorption on chitosan and derivative materials: a strategy for polymer modification and optimum use. React Funct Polym 60:137–149
3. Chen SD, Hsieh JF, Tsai SC, Lin WY, Cheng KY, Wang SJ (2001) Intratumoural injection of ^{90}Y microspheres into an animal model of hepatoma. Nucl Med Commun 22:121–125
4. DiGiovanni J, Rymer J, Slaga TJ, Boutwell RK (1982) Anticarcinogenic and cocarcinogenic effects of benzo[e] pyrene and dibenz[a,c]anthracene on skin tumor initiation by polycyclic hydrocarbons. Carcinogenesis 3:371–375
5. Graham MC, Pentlow KS, Mawlawi O, Finn RD, Daghighian F, Larson SM (1997) An investigation of the physical characteristics of ^{66}Ga as an isotope for PET imaging and quantification. Med Phys 24:317–326
6. Guibal E (2003) Interactions of metal ions with chitosan--based sorbents. Sep Purif Technol 38:43–74
7. Hamoudeh M, Fessi H, Salim H, Barbos D (2008) Holmium-loaded PLLA nanoparticles for intratumoral radiotherapy via the TMT technique: preparation, characterization, and stability evaluation after neutron irradiation. Drug Dev Ind Pharm 34:796–806
8. Holte O, Skretting A, Bach-Gansmo T *et al*. (2006) Localized internal radiotherapy with ^{90}Y particles embedded in a new thermosetting alginate gel: a feasibility study in pigs. Nucl Med Commun 27:185–190
9. ICRU (1984) Stopping powers for electrons and positrons. ICRU Report 37. International Commission on Radiation Units and Measurements, Bethesda, USA
10. Inoue K, Yoshizuka K, Ohto K (1999) Adsorptive separation of some metal ions by complexing agent types of chemically modified chitosan. Anal Chim Acta 388:209–218
11. Jalilian AR, Akhlaghi M, Shirazi B *et al*. (2006) [^{201}Tl] (III)-Bleomycin for tumor imaging. Radiochim Acta 94:453–459
12. Jalilian AR, Rowshanfarzad P, Yari-Kamrani Y, Shafaii K (2007) Production and tumor uptake of [^{64}Cu]pyruvaldehyde-bis(N4-methylthiosemicarbazone) for PET and/or therapeutic purposes. Nucl Med Rev 10:6–11
13. Junfeng Y, Duanzhi Y, Xiaofeng M *et al*. (1999) [^{188}Re] Rhenium sulfide suspension: a potential radiopharmaceutical for tumor treatment following intratumor injection. Nucl Med Biol 26:573–579
14. Junfeng Y, Duanzhi Y, Xiaofeng M *et al*. (1999) Preparation of [^{188}Re]rhenium sulfide suspension and its biodistribution following intratumor injection in mice. J Labelled Compd Rad 42:233–243
15. Lewis MR, Reichert DE, Laforest R *et al*. (2002) Production and purification of gallium-66 for preparation of tumor-targeting radiopharmaceuticals. Nucl Med Biol 29:701–706
16. Li Q, Dunn ET, Grandmaison EW, Goosen MFA (1996) Applications and properties of chitosan. In: Goosen MFA (ed) Applications of chitin and chitosan. CRC Press, Florida, pp 3–30
17. Lin WY, Tsai SC, Hsieh JF, Wang SJ (2000) Effects of ^{90}Y microspheres on liver tumors: comparison of intratumoral injection method and intraarterial injection method. J Nucl Med 41:1892–1897
18. Moadel RM, Weldon RH, Katz EB *et al*. (2005) Positherapy: targeted nuclear therapy of breast cancer with ^{18}F-2-deoxy-2-fluoro-D-glucose. Cancer Res 65:698–702
19. Navarro R, Guzman J, Saucedo I, Revilla J, Guibal E (2003) Recovery of metal ions by chitosan: sorption mechanisms and influence of metal speciation. Macromol Biosci 3:552–561
20. Pal PB, Varshney VP, Gupta DK (1986) Total stopping power formulae for high energy electrons and positrons. Nucl Instrum Methods B 16:1–4
21. Park KB, Kim YM, Kim JR (1998) Radioactive chitosan complex for radiation therapy. US Patent no. 5762903
22. Park KB, Kim YM, Shin BC, Kim JR (1996) Study on the preparation of new ^{166}Ho-chitosan complex and its macroaggregates for a potential use of internal radiotherapy. Kor J Nucl Med 30:351–360
23. Park KB, Kim YM, Shin BC, Kim JR, Ryu JM, Lim SM (1998) Therapeutic application of new holmium-166 chitosan complex in malignant and benign diseases. IAEA--TECDOC-1029. IAEA, Vienna, pp 569–580
24. Pimblott SM, LaVerne JA, AlbaGarcia A, Siebbeles LDA (2000) Energy loss by nonrelativistic electrons and positrons in polymers and simple solid hydrocarbons. J Chem Phys B 104:9607–9614
25. Pimblott SM, Siebbeles LDA (2002) Energy loss by non--relativistic electrons and positrons in liquid water. Nucl Instrum Methods B 194:237–250
26. Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31:603–632
27. Sabet M, Rowshanfarzad P, Jalilian AR, Ensaf MR, Rajamand AA (2006) Production and quality control of ^{66}Ga radionuclide. Nukleonika 51;3:147–154
28. Seltzer SM, Berger MJ (1982) Evaluation of the collision stopping power of elements and compounds for electrons and positrons. Int J Appl Radiat Isot 33:1189–1218
29. Shin BC, Park KB, Jang BS, Lim SM, Shim CK (2001) Preparation of ^{153}Sm-chitosan complex for radiation synovectomy. Nucl Med Biol 28:719–725
30. Suzuki YS, Momose Y, Higashi N *et al*. (1998) Biodistribution and kinetics of holmium-166-chitosan complex (DW-166HC) in rats and mice. J Nucl Med 39:2161–2166
31. Tuck DG (1993) The coordination and solution chemistry of aluminium, gallium, indium and thallium. In: Downs AJ (ed) Chemistry of aluminum, gallium, indium and thallium. Blackie Academic and Professional, London, pp 430–473
32. Ugur O, Kothari PJ, Finn RD *et al*. (2002) Ga-66 labeled somatostatin analogue DOTA-DPhe1-Tyr3-octreotide as a potential agent for positron emission tomography imaging and receptor mediated internal radiotherapy of somatostatin receptor positive tumors. Nucl Med Biol 29:147–157
33. Varma AJ, Deshpande SV, Kennedy JF (2004) Metal complexation by chitosan and its derivatives. Carbohyd Polym 55:77–93
34. Wang XM, Yin ZY, Yu RX, Peng YY, Liu PG, Wu GY (2008) Preventive effect of regional radiotherapy with phosphorus-32 glass microspheres in hepatocellular carcinoma recurrence after hepatectomy. World J Gastroenterol 14:518–523
35. Weiner RE, Thakur ML (2003) Chemistry of gallium and indium radiopharmaceuticals. In: Welch MJ, Redvanly CS

(eds) Handbook of radiopharmaceuticals: radiochemistry and applications. John Wiley and Sons, Chichester, pp 363–400

36. Zweit J, Sharma H, Downey S (1987) Production of gallium-66, a short-lived positron emitting radionuclide. Int J Rad Appl Instrum A 38:499–501