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Metallofullerene drug design☆

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Contents

Abstract

Endohedral lanthanide metallofullerenes are new molecules that may have useful medicinal properties. In particular, endohedral holmium metallofullerenes have been utilized in a model metallofullerene radiotracer study. The ^{165}Ho metallofullerenes were chemically functionalized to impart water solubility and then neutron activated to ^{166}Ho in order to determine their biodistribution and metabolism properties. The results have been evaluated for poten-

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tial applications of lanthanide metallofullerenes as new diagnostic or therapeutic radiopharmaceuticals. Use of metallofullerenes in conventional diagnostic radiology (MRI contrast and X-ray imaging agents) has also been considered.

Keywords: Contrast agents; Endohedral metallofullerenes; Fullerene biodistribution; Metallofullerols; Nuclear medicine

1. Introduction

Potential applications for fullerene and metallofullerene materials have been widely proposed since their discovery in 1985, but despite intense inquiry, no fullerene-based products have been developed to date. Biomedical applications are one field that holds special promise for yielding viable fullerene-based products, since relatively small quantities of material are required for effective dosing. Studies directed toward this end have demonstrated beneficial in vitro biological properties for empty C_{60} and its organic-functionalized derivatives [1–9]. Several reports have also recently shown that the fullerene carbon cage is relatively non-toxic and not metabolized in vivo [10–13]. Biodistribution studies of C_{60}, its water-soluble derivatives, and one metallofullerene ($Gd@C_{82}$) have indicated that these carbon cage compounds primarily localize in the liver and clear only very slowly [5,14–17]. Nevertheless, the non-toxicity of the carbon cage makes metallofullerene-based drugs potentially feasible for medicinal applications. The internal metal of an endohedral metallofullerene is effectively isolated from its surrounding environment, giving the metallofullerene a distinct advantage over the metal chelate complexes that are commonly used in radiomedicine and diagnostic radiology. These chelate complexes are prone to in vivo metal dissociation, but no such release of free metal is possible from an unmetabolized metallofullerene [18,19]. There is substantial overlap of known endohedral metallofullerenes with metal isotopes having established or potential use in nuclear medicine (see Fig. 1), making the metallofullerene ideally suited for development as a general radioisotope-delivery system. Utilization of these unique compounds as MRI contrast or X-ray imaging agents further broadens the scope of possible medicinal applications. Recent work toward development of holmium metallofullerene-based radiopharmaceuticals is described below [20–24].

2. Experimental

Holmium metallofullerenes were prepared and separated from empty fullerenes using previously described carbon-arc and HPLC techniques [20,21]. Water-soluble holmium metallofullerols were prepared under alkaline phase-transfer conditions [21,25]. Metallofullerene neutron irradiations were conducted at the Missouri

University Research Reactor or the HFIR high-flux isotope reactor (Oak Ridge National Laboratory) under conditions that have been described in detail elsewhere [20–24].

Biodistribution and metabolism studies of the holmium metallofullerol compound were performed in BALB/c mice and Fischer rats, respectively [21]. ^{166}Ho (ca. 10 μCi activity) was injected into each animal for in vivo testing. Biodistribution and metabolism studies were conducted under approved protocols of the Institutional Animal Care and Use Committee at the Oak Ridge National Laboratory.

3. Results and discussion

3.1. Water-soluble metallofullerene derivatives

One of the main challenges in developing fullerene-based drugs is the task of making them water soluble for in vivo use. The non-functionalized fullerene cages are soluble only in non-polar organic solvents, making them incompatible with biological systems. Fortunately, the exterior of the fullerene carbon cage has a rich synthetic organic chemistry, which allows a wide variety of water-soluble derivatives to be prepared. Synthetic versatility of the carbon scaffold might eventually allow fullerene-based drugs to be directed to specific organ sites through preparation of derivatives having tissue-targeting vectors.

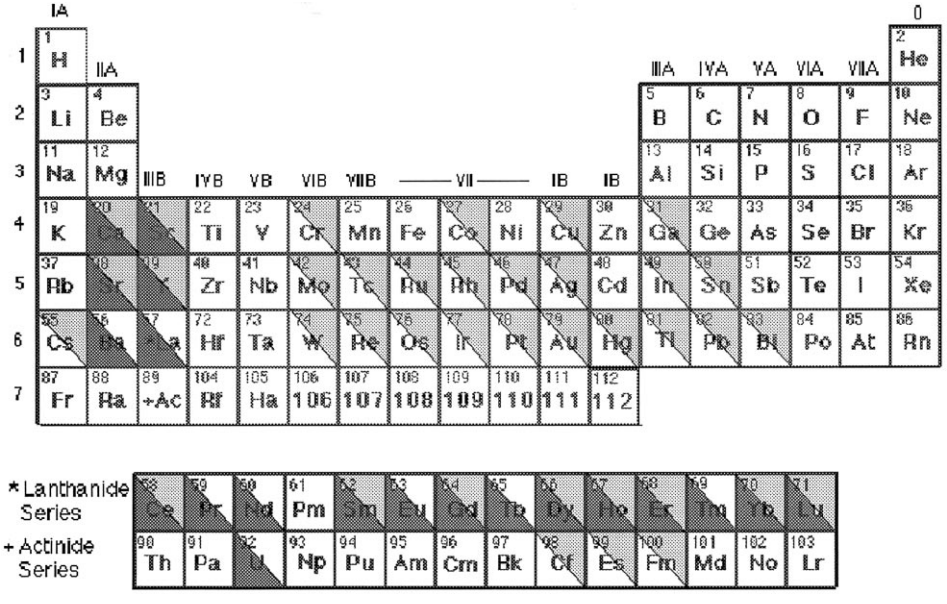

Fig. 1. Metals capable of forming endohedral metallofullerenes (dark shading) and those useful for nuclear medicine (light shading).

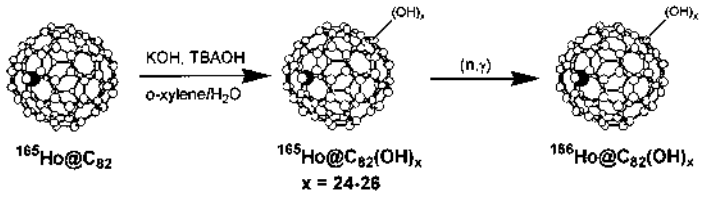

Fig. 2. Scheme employed for preparing water-soluble ^{166}Ho metallofullerols.

For the present biodistribution study, a simple holmium metallofullerol, (i.e. $Ho@C_{82}(OH)_x$), was chosen. This particular water-soluble derivative was selected because it is formed in very high yield, and the polyalcohol groups are compatible with high neutron-flux conditions. The scheme for preparation of the ^{165}Ho metallofullerol and the subsequent transformation of the internal metal to the ^{166}Ho radionuclide is illustrated in Fig. 2. While a wide variety of water-solubilizing substituents may be attached to the fullerene, they must not include atoms that activate in addition to the metal center (for instance, S or P). The strategy of preparing the water-soluble metallofullerene derivative before irradiation avoids having to perform involved chemical manipulations on very small quantities of 'hot' irradiated material.

3.2. Behavior of metallofullerenes in neutron fluxes

A fundamental question that must be addressed before metallofullerenes can be employed as radiopharmaceuticals is whether they efficiently survive neutron bombardment to become radioactive. During neutron irradiation, metallofullerenes can either be activated to the internal radionuclide species or degraded by neutrons through two independent routes (see Fig. 3) [20,22–24]. Fast neutrons (>1000 eV) can disrupt the cage integrity when the high energy particles translocate carbon atoms from their fixed positions in the fullerene cage lattice. Cumulative carbon-atom translocations result in the eventual release of the internal metal from the metallofullerene. The metal can also escape from the carbon cage by a recoil process during (n, γ) activation. When an atom absorbs a neutron to become radioactive, it immediately emits a prompt γ-ray, and conservation of momentum results in recoil of the newly formed radionuclide. For ^{166}Ho, this recoil energy is ≈ 125 eV, which is much greater than the fullerene carbon–carbon bond energy (3–7 eV per bond) [26,27], and it represents a potentially serious impediment toward developing metallofullerene-based radiopharmaceuticals. Recoil also occurs during β-decay, but the recoil energies are much lower (tens of eVs). The neutron-irradiation events shown in Fig. 3 are presented for an underivatized metallofullerene, but they are equally valid for water-soluble metallofullerene derivatives, such as a metallofullerol.

We have previously demonstrated that holmium metallofullerenes are degraded primarily by fast neutrons in moderately thermal-neutron fluxes, but under highly thermal flux conditions, prompt γ-recoil is the only operative mechanism [20,22–

24]. Stability of Gd metallofullerenes against β-recoil has been noted by others [28]. Metallofullerene survival is maximized (30%) under highly thermal irradiation conditions, and the survival rate remains nearly constant with time. The constant survivability in highly thermal-neutron fluxes permits the metallofullerene material to be irradiated for many hours at a time, allowing high activities of radioactive metallofullerene species to be produced.

3.3. Biodistribution and metabolism studies of holmium metallofullerols

Holmium metallofullerenes were specifically chosen for exploratory neutron-irradiation and biodistribution studies because of the natural monoisotopic character and high activation cross-section ($\sigma = 58.1$ barns) of ^{165}Ho and the established use of activated ^{166}Ho in nuclear medicine [29–31]. The biodistribution of the ^{166}Ho metallofullerol was followed over 48 h. At intervals of 1, 4, 24, and 48 h mice injected with the ^{166}Ho radionuclide were sacrificed, and the following tissues were harvested for analysis: muscle (thigh), bone, skin, uterus/ovaries, large intestine, stomach (emptied), liver, kidneys, spleen, fat (abdominal), thymus, heart, lungs, brain, and blood. The results of the biodistribution study are presented in Fig. 4 [21].

The metallofullerol biodistribution differs significantly from that of other derivatized fullerenes, as well as that of a simple holmium chelate compound,

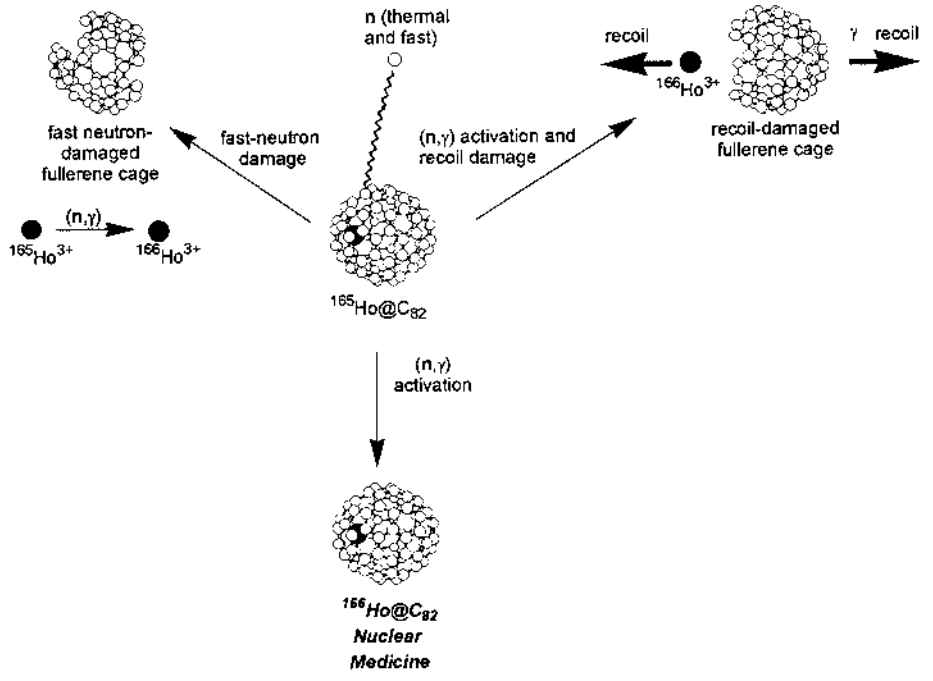

Fig. 3. Possible outcomes of irradiating $^{165}Ho@C_{82}$ in a thermal/fast-neutron flux.

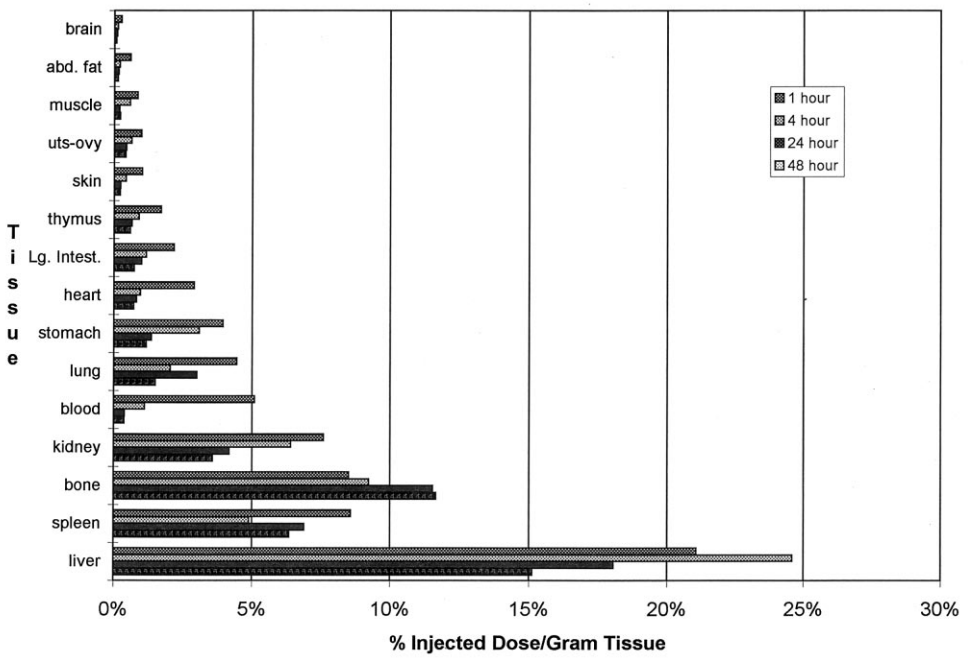

Fig. 4. Biodistribution data for $^{166}Ho@C_{82}(OH)_x$ in BALB/c mice. Reproduced with permission from [21].

$Na_2[^{166}Ho(DTPA)\cdot H_2O]$. The ^{166}Ho DTPA complex was chosen as a control because of its similarity to commercially-used Gd^{3+} MRI contrast agents employing the same ligand. Near quantitative excretion of $Na_2[^{166}Ho(DTPA)\cdot H_2O]$ was observed in less than 1 h after administration, in good agreement with literature biodistribution data for the Gd analog [32]. In contrast, the metallofullerol was widely distributed throughout the body, except for tissues having limited blood flow, such as the brain and fat. A significant percentage of the total activity was retained throughout the 48 h of the study, particularly in the kidneys, bone, spleen, and liver. All tissues displayed a slow clearance of ^{166}Ho over 48 h, except for bone which showed gradually increasing localization with time.

These results are significant because they suggest that fullerenes and metallofullerenes can be selectively tissue-targeted through choice of the water-solubilizing substituent. Previous biodistribution studies of water-soluble fullerene derivatives have demonstrated primary localization in the liver (>90–95%), with only very slow clearance observed (>1 week) [5,14–17]. While liver localization is still significant for the metallofullerol, the accumulation is much lower, and the slow clearance over 48 h is much more rapid than that noted previously. The localization of the metallofullerol in bone suggests that this metallofullerene species is selectively targeted for this tissue and might find a specialty niche as a chemotherapy agent for treating leukemia, bone cancer, or bone pain. Similar polyhydroxylated compounds also demonstrate a high affinity for cortical bone [33,34].

Metabolism studies also indicated that the fullerol cage was not likely degraded under biological conditions [21]. Analysis of the ^{166}Ho activity extracted from the livers of the BALB/c mice showed that the extractable activity was $>95\%$ non-ionic. This behavior indicates that the holmium atom is maintained within the fullerol cage. The excreted activity appears primarily in the urine within 24 h, which explains the rapid buildup and then drop of the activity in the kidney. Beyond this time, only about 1.5% of the activity was excreted per day, with nearly equal activities located in the feces and urine. Slow excretion of a water-soluble C_{60} derivative has been linked to in vivo protein binding [17], and this effect may also cause slow excretion of the metallofullerol. Protein binding is also one possible explanation for the short-term metallofullerol accumulation in blood-rich tissues, while the eventual localization in bone is due to 'selective' tissue targeting.

4. Conclusions

Water-soluble holmium metallofullerenes have been neutron irradiated and employed in a radiotracer biodistribution study for the first time [21]. This work has demonstrated a biodistribution different than those of other derivatized fullerenes, and the results indicate that the fullerene cage can be selectively tissue-targeted through choice of the water-solubilizing substituent. The non-toxicity and improved clearance of the metallofullerol suggest that metallofullerenes merit further study for nuclear medicine and diagnostic radiology applications. Use of metallofullerenes for the latter purpose would center chiefly on their MRI contrast and X-ray imaging properties. Metallofullerene MRI contrast agents could take advantage of the vast paramagnetic cage surface area (200 Å^2) [35], while X-ray imaging agents would utilize the scattering effects of the internal heavy metal atom(s) [36]. We are currently exploring both of these realms, in addition to continuing the development of metallofullerene-based radiopharmaceuticals.

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References

[1] R.F. Schinazi, L.Y. Chiang, L.J. Wilson, D.W. Cagle, in: K.M. Kadish, R.S. Ruoff (Eds.), Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials, vol. 4, The Electrochemical Society, Pennington, NJ, 1997, pp. 357–360.

[2] S.H. Friedman, P.S. Ganapathi, Y. Rubin, G.L. Kenyon, J. Med. Chem. 41 (1998) 2442.
[3] D.I. Schuster, S.R. Wilson, R.F Schinazi, Bioorg. Med. Chem. 6 (1996) 1253.
[4] S.H. Friedman, D.L. DeCamp, R.P. Sijbesma, G. Srdanov, F. Wudl, G.L. Kenyon, J. Am. Chem. Soc. 115 (1993) 6506.
[5] R.F. Schinazi, R. Sijbesma, G. Srdanov, C.L. Hill, F. Wudl, Antimicrob. Agents Chemother. 37 (1993) 1707.
[6] L.Y. Chiang, F.-J. Lu, J.-T. Lin, in: K.M. Kadish, R.S. Ruoff (Eds.), Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials, vol. 2, The Electrochemical Society, Pennington, NJ, 1995, p. 699.
[7] L.L. Dugan, J.K. Gabrielsen, S.P. Yu, T.-S. Lin, D.W. Choi, Neurobiol. Dis. 3 (1996) 129.
[8] H. Tokuyama, S. Yamago, E. Nakamura, T. Shiraki, Y. Sugiura, J. Am. Chem. Soc. 115 (1993) 7918.
[9] A.S. Boutorine, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura, C. Hélène, Angew. Chem. Int. Ed. Engl. 33 (1994) 2462.
[10] M.A. Nelson, F.E. Domann, G.T. Bowden, S.B. Hooser, Q. Fernando, D.E. Carter, Toxicol. Ind. Health. 9 (1993) 623.
[11] R. Bullard-Dillard, K.E. Creek, W.A Scrivens, J.M. Tour, Bioorg. Chem. 24 (1996) 376.
[12] H.H.C. Chen, C. Yu, T.H. Ueng, S. Chen, B.J. Chen, K.J. Huang, L.Y. Chiang, Toxicol. Pathol. 26 (1998) 143.
[13] F. Moussa, M. Pressac, M. Hadchouel, B. Arbeille, P. Chrétien, F. Trivin, R. Céolin, H. Szwarc, in: K.M. Kadish, R.S. Ruoff (Eds.), Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials, vol. 5, The Electrochemical Society, Pennington, NJ, 1997, pp. 332–336.
[14] F. Moussa, F. Trivin, R. Céolin, M. Hadchouel, P.Y. Sizaret, V. Greugny, C. Fabre, A. Rassat, H. Szwarc, Fullerene Sci. Technol. 4 (1996) 21.
[15] K. Kobayashi, M. Kuwano, K. Sueki, K. Kikuchi, Y. Achiba, H. Nakahara, N. Kananishi, M. Wantanabe, K. Tomura, J. Radioanal. Nucl. Chem. 192 (1995) 81.
[16] S. Yamago, H. Tokuyama, E. Nakamura, K. Kikuchi, S. Kananishi, K. Sueki, H. Nakahara, S. Enemoto, F. Ambe, Chem. Biol. 2 (1995) 385.
[17] P. Rajagopalan, F. Wudl, R.F. Schinazi, F.D. Boudinot, Antimicrob. Agents Chemother. 40 (1996) 2262.
[18] S. Jurisson, D. Berning, W. Jia, D. Ma, Chem. Rev. 93 (1993) 1137.
[19] E.D. Unger, K. Shen, G. Wu, T. Fritz, Mag. Res. Med. 22 (1991) 304.
[20] D.W. Cagle, T.P. Thrash, M. Alford, L.P.F. Chibante, G.J. Ehrhardt, L.J. Wilson, J. Am. Chem. Soc. 118 (1996) 8043.
[21] D.W. Cagle, Thesis, Rice University, Houston, TX, 1998.
[22] T.P. Thrash, D.W. Cagle, J.M. Alford, G.J. Ehrhardt, S. Mirzadeh and L.J. Wilson, Chem. Phys. Lett. 308 (1999) 329.
[23] T.P. Thrash, D.W. Cagle, M. Alford, G.J. Ehrhardt, J.C. Lattimer, L.J. Wilson, in: K.M. Kadish, R.S. Ruoff (Eds.), Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials, vol. 4, The Electrochemical Society, Pennington, NJ, 1997, pp. 349–356.
[24] D.W. Cagle, T.P. Thrash, L.J. Wilson, G.J. Ehrhardt, M. Alford, L.P.F. Chibante, in: K.M. Kadish, R.S. Ruoff Jr. (Eds.), Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials, vol. 3, The Electrochemical Society, Pennington, NJ, 1996, p. 854.
[25] J. Li, A. Takeuchi, M. Ozawa, X. Li, K. Saigo, K. Kitazawa, J. Chem. Soc. Chem. Comm. (1993) 1784–1785.
[26] M. Saunders, H.A. Jiménez-Vázquez, R.J. Cross, R.J. Poreda, Science 259 (1993) 1428.
[27] R.L. Murry, G.E. Scuseria, Science 261 (1994) 791.
[28] K. Kikuchi, K. Kobayashi, K. Sueki, S. Suzuki, H. Nakahara, Y. Achiba, K. Tomura, M. Katada, J. Am. Chem. Soc. 116 (1994) 9775.
[29] R.J. Mumper, U.Y. Ryo, M. Jay, J. Nucl. Med. 32 (1991) 2139.
[30] E. Dadachova, S. Mirzadeh, R.M. Lambrecht, E.L. Hetherington, F.F Knapp Jr., Anal. Chem. 66 (1994) 4272.

[31] W.K.A. Louw, I.C. Dormehl, A.J. van Rensburg, N. Hugo, A.S. Alberts, O.E. Forsyth, G. Beverley, M.A. Sweetlove, J. Marais, M.G. Lötter, A. van Aswegen, Nucl. Med. Biol. 23 (1996) 935.
[32] F.S. Prato, G. Wisenberg, T.P. Marshall, P. Uksik, P. Zabel, J. Nucl. Med. 29 (1988) 1683.
[33] T.M. Willson, P.S. Charifson, A.D. Baxter, N.G. Geddie, Bioorg. Med. Chem. Lett. 6 (1996) 1043.
[34] T.M. Willson, B.R. Henke, T.M. Momtahen, D.T. Garrison, L.B. Moore, N.G. Gettie, P.G. Baer, Bioorg. Med. Chem. Lett. 6 (1996) 1047.
[35] D.W. Cagle, J.M. Alford, J. Tien, L.J. Wilson, in: K.M. Kadish, R.S. Ruoff (Eds.), Fullerenes: Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials, vol. 4, The Electrochemical Society, Pennington, NJ, 1997, pp. 361–368.
[36] H.N. Cardinal, D.W. Holdsworth, M. Drangova, B.H. Hobbs, A. Fenster, Med. Phys. 20 (1993) 15.