Measurement of x-ray attenuation coefficients of aqueous solutions of indocyanine green and glycated chitosan

Fang Xu and Hong Liu[a)]
Department of Radiology and Biomedical Engineering, University of Virginia, Charlottesville, Virginia 22908

Xizeng Wu
Department of Radiology, The University of Alabama at Birmingham, Birmingham, Alabama

Hangyi Jiang
Department of Radiology and Biomedical Engineering, University of Virginia, Charlottesville, Virginia 22908

Robert E. Nordquist
Wound Healing of Oklahoma, Incorporated

Wei R. Chen
Department of Physics and Astronomy, University of Oklahoma and Department of Physics, Oklahoma School of Science and Mathematics

(Received 21 September 1998; accepted for publication 21 April 1999)

We report our experimental results of measurements of x-ray attenuation coefficients of aqueous solutions of a light absorbing dye, indocyanine green, and an immunoadjuvant, glycated chitosan. In the treatment of metastatic tumors in rats using a novel laser immunotherapy these solutions were administered *in situ*. The x-ray attenuation data of the solutions are essential to development of an x-ray digital imaging system for monitoring the administration of the solution, as well as for the distribution and the diffusion of the solution in tumors and in surrounding tissue. The composition of the solutions, the measurement system configuration, and the technique used to determine the attenuation coefficients are described. The experimental results show that glycated chitosan has a higher attenuation coefficient compared to indocyanine green and water. Our experimental data proved that, even at low concentrations, the x-ray attenuation through these aqueous solutions could be differentiated. Therefore, a digital x-ray imaging technique can be used effectively in monitoring and controlling the intratumor diffusion and distributions of these solutions. © *1999 American Association of Physicists in Medicine.* [S0094-2405(99)01307-3]

Key words: x-ray attenuation coefficients, CCD imaging system, indocyanine green, glycated chitosan

I. INTRODUCTION

To achieve selective photothermal destruction of deep tumors, a light absorbing dye, indocyanine green (ICG), has been used in conjunction with a near-infrared laser. Through intratumor injection, ICG molecules can be strategically placed in a tumor and when they absorb a penetrating light, such as an 805 nm laser light, selective photothermal tumor tissue destruction results.[1–3] A novel immunoadjuvant, glycated chitosan (GC), has also been used with ICG to induce an antitumor immune response in the treatment of metastatic tumors by combining the selective photothermal interaction with a specifically targeted immunological reaction.[4–7] Since ICG and GC were administered by direct intralesional injection, their distribution and dynamic diffusion in tissue are crucial in determining the outcomes of treatment. A digital x-ray technique showed the ability to monitor and guide the administration and intratumor diffusion of ICG and GC.[8] However, the x-ray attenuation coefficients of ICG and GC are not available. The objective of this research was to study the x-ray transmission through these solutions, in comparison with that through water, in order to determine whether our current technique can differentiate various solutions at the concentrations used in laser immunotherapy treatment. The measurement procedure and experimental results are reported here.

II. MATERIALS AND METHODS

A. Composition of the solutions

In our experiments, x-ray transmission data were collected for four different solutions, which were used in the laser immunotherapy for cancer treatment in animal studies. Their compositions are the following: water (H_2O); aqueous solution of 0.5% ICG ($C_{43}H_{47}N_2NaO_6S_2$); aqueous solution of 1% GC*; mixture of ICG and GC (0.5% ICG and 1% GC).

ICG was purchased from Becton-Dickinson Co., Cockeysville, MD. It is a sterile, water soluble, tricarbocyanine dye with a molecular weight of 775. ICG has been used clinically for diagnostics and treatment. GC is a derivative of chitosan. It is a biopolymer with a molecular weight of approximately 1.5 million.

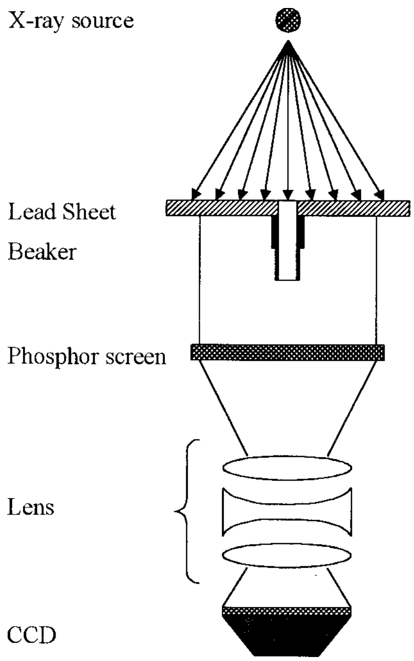

FIG. 1. Apparatus for measuring x-ray attenuation coefficients using an optically coupled CCD x-ray imaging system. The beaker containing the aqueous solutions is placed 13 cm above the scintillating screen, and below the x-ray source. A piece of lead plate, with a 10.00 mm×6.00 mm opening in the middle, was used to limit the size of the x-ray beam. The opening of the lead plate was aligned with the beaker. The transmitted x-ray signals are stored in a computer for later analysis.

B. System configuration

A prototype optically coupled charge coupled device (CCD) x-ray imaging system was used to measure x-ray attenuation.[9–11] Figure 1 shows the configuration of the apparatus. The system, consisting of a scintillating screen (Min-R Medium, Kodak, NY), a custom relay lens, and a CCD camera (Photometrics, AZ), is attached to a clinical mammographic x-ray machine (GE 600T, Mo target and Mo filtration). The x-ray source was operated with a 0.3 mm focal spot size. The camera employs a mechanical shutter. An optical hood was used to shield the CCD sensor from ambient light. The CCD array has 1024×1024 pixels, 14 bit digitization, and each pixel measures 0.024 mm×0.024 mm. The quantum efficiency of the CCD image is about 70%–75% at 550 nm (backilluminated CCD). The camera was operated at a temperature of −25 °C to reduce electron noise. The low temperature was achieved with a compact thermoelectric cooler.

During the measurement, a plastic beaker (that contains the aqueous solutions) is placed 13 cm above the scintillating screen, and below the x-ray source. The overall distance between the x-ray source and the scintillator was 65 cm. The inner size of the beaker is 10.20 mm×6.20 mm, and the bottom of the beaker is 0.88 mm thick. To further reduce the effect of scattered radiation, a piece of lead plate, with a 10.00 mm×6.00 mm opening in the middle, was used to limit the size of the x-ray beam. As shown in Fig. 1, the opening of the lead plate was aligned with the beaker.

The operation of the system is described as follows: during x-ray exposure, the x-ray beam passes through the aqueous solutions contained in the beaker, is attenuated, and then interacts with the scintillating screen. There the x-ray photons are converted into a large number of visible light photons. The relay lens then projects the optical signal from the scintillating screen to the photosensitive surface of the CCD camera. The CCD pixel array samples the information and creates a digital image. The quantitative value of the recorded signal is obtained by measuring the mean pixel value of a specific area (region of interest) of the digital images.

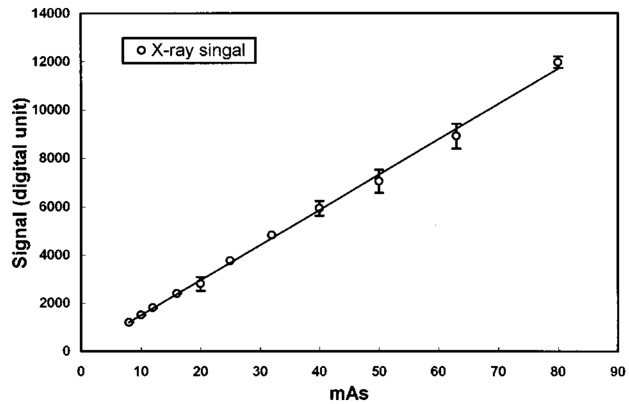

FIG. 2. X-ray signal vs mA at 22 kVp. The error bars are standard deviations. The solid line is the best linear fit. The data verify that the CCD system responds linearly to x-ray exposure.

C. Technique for determining the attenuation coefficients

The attenuated x-ray photon flux obeys Beer's law: $I = I_0 e^{-\alpha t}$, where t is depth of the solution, α is the attenuation coefficient of the solution, I_0 is the photon flux when $t = 0$ cm, and I is the photon flux after passing through the solution of t depth. To determine α, the x-ray signals transmitted through the solution at depths of $t = 0$, 0.5, 1, and 1.5 cm were measured. At each depth, five measurements were

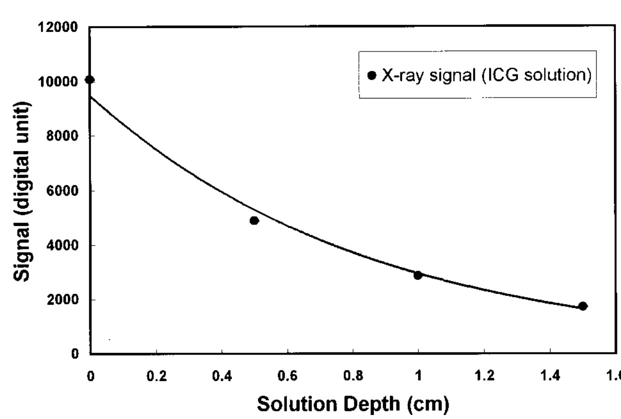

FIG. 3. X-ray transmission data through indocyanine green solution (0.5%) at different depths. The x-ray exposure is 22 kVp. The solid line is the best exponential fit that gives rise to a coefficient of attenuation of 1.1709 cm^{-1}. The standard deviations are too small to show.

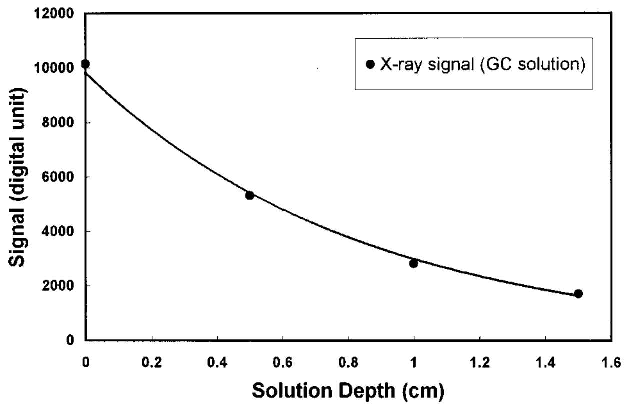

FIG. 4. X-ray transmission data through glycated chitosan solution (1%) at different depths. The x-ray exposure is 22 kVp. The solid line is the best exponential fit that gives rise to a coefficient of attenuation of 1.1925 cm^{-1}. The standard deviations are too small to show.

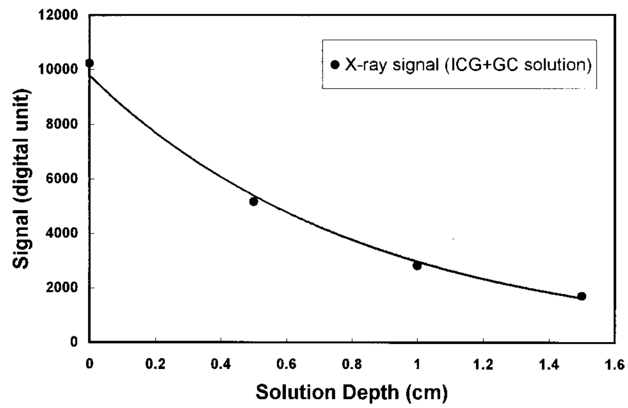

FIG. 5. X-ray transmission data through a solution of indocyanine green and glycated chitosan formulation (0.5% ICG and 1% GC) at different depths. The x-ray exposure is 22 kVp. The solid line is the best exponential fit that gives rise to a coefficient of attenuation of 1.1954 cm^{-1}. The standard deviations are too small to show.

made. Using Beer's law, an exponential curve fitting was used to obtain the values of the attenuation coefficients.

D. Half value layer measurement

The x-ray attenuation coefficients reported in this article were measured under practical conditions. Namely, they were not measured under a monoenergetic x-ray beam; rather, these are x-ray attenuation coefficients relative to the x-ray spectra that were used in monitoring the intratumor distribution of the aqueous solutions in our practical application.[8]

To quantify the spectral composition of the beam, the half value layer (HVL) and the second HVL were measured with a set of high purity (99.99%) aluminum filters (0.1 mm in thickness) and an x-ray exposure meter (Rad Check™ Plus, model 06-526, Nuclear Associates). When measuring the HVL, the beaker that contains aqueous solutions (water+GC) was removed from the path of the beam. The beam hardening effects caused by the aqueous solutions were neglectable, because of the specific application of the measured results, and also because the variation of the depth of the solutions is small (0.5–1.5 cm).

III. RESULTS

The measured mean pixel values ranged from 1950 to 13 500 when the x-ray exposure varied from 8 to 80 mA and from 22 to 30 kVp. In such a range, the CCD system linearly responds to x-ray exposures, as verified by the measured results given in Fig. 2.

Figures 3–5 show the x-ray signals through different solutions up to a 1.5 cm depth at 22 kVp. The data were used to determine the attenuation coefficients using an exponential fit. The transmitted signals through 0.5% ICG solution are given in Fig. 3 and α was determined as 1.1709 cm^{-1}. The signals through the 1% GC solution are given in Fig. 4 with a determined α value of 1.1925 cm^{-1}. The x-ray signals through the mixture of ICG+GC (0.5% and 1%, respectively) are shown in Fig. 5 and a slightly increased α (1.1954 cm^{-1}) was obtained.

When the energy of the x ray was increased, the attenuation coefficients of all the solutions decreased. The x-ray attenuation coefficients of four different solutions at 22, 26, and 30 kVp, are summarized in Table I. The standard deviations of the measurement results are also presented in Table I.

IV. DISCUSSION AND CONCLUSIONS

Intratumor injection of anticancer drugs may be a plausible treatment modality because of direct targeting of the tumor cells. The intratumor administration of indocyanine green and glycated chitosan in the novel laser immunotherapy has achieved the desired effect: precise tumor tissue destruction and induction of host immune responses. Specifically, ICG has served as a light absorbing dye in the tumor mass and GC has served as an immune stimulant. The combined photothermal and photoimmunological reactions have eradicated metastatic tumors in rats.[4–7] Apparently the outcome of the laser immunotherapy depends, at least partially,

TABLE I. Attenuation coefficients of different solutions α (1/cm) at three different x-ray energy levels.

Exposure (kVp)	HVL (mm)	Second HVL (mm)	Water	ICG	GC	ICG+GC
22	0.2250	0.3250	1.1617±0.0003	1.1709±0.0005	1.1925±0.0007	1.1954±0.0013
26	0.2976	0.3774	1.0365±0.0011	1.0462±0.0010	1.0618±0.0005	1.0915±0.0005
30	0.3268	0.4005	0.9709±0.0011	0.9803±0.0009	1.0233±0.0002	1.0496±0.0006

on the administration and distribution of the injected solutions. Furthermore, the dynamic diffusion of the solutions into the tumor and surrounding tissue is also crucial in the real-time monitoring of the laser treatment. The x-ray attenuation coefficients of these solutions are important data if the x-ray imaging technique is to be used for such monitoring purposes. This current project was designed to use a prototype x-ray imaging system to measure the attenuation coefficients.

The digital x-ray imaging system, shown schematically in Fig. 1, is a reliable system. It showed a linear response to the x-ray exposures used in our measurements and is shown in Fig. 2. The x-ray signal measurement for different solutions also showed the stability of the system. The standard deviations of the measurement at each depth was very small (less than 0.2% of the measured values of the transmitted signals).

The data in Table I show that the attenuation coefficients of these four solutions are close to one another. However, there still exist slight differences among them. Their order is $\alpha_{\mathrm{Water}}<\alpha_{\mathrm{ICG}}<\alpha_{\mathrm{GC}}<\alpha_{\mathrm{ICG+GC}}$. The molecular weights of ICG and GC are much larger than that of water, and their attenuation coefficients are expected to be larger than that of water. However, because the concentrations of ICG and/or GC were very low in the solutions investigated (0.5% ICG and 1% GC), their x-ray attenuation coefficients were expected to be close to that of water. Our experimental results verified these expectations, as shown by the data in Table I. The data also indicated that, even at such low concentrations, the difference in x-ray attenuation through different solutions could be determined. Therefore, the digital x-ray imaging technique can be used effectively in monitoring and controlling the intratumor diffusion and distributions of these solutions.

Furthermore, the x-ray attenuation was decreased when the x-ray energy was increased, as shown in Table I. With a change of 4 kVp, the attenuation coefficients for the four different solutions varied between 4% and 10%, a level that can be easily measured using the current technique. This coefficient change could be used when more delicate differentiation is needed.

In summary, our experimental results showed that the x-ray imaging technique is capable of differentiating different solutions at low concentrations, thereby showing the feasibility of monitoring intratumor drug administration.

ACKNOWLEDGMENTS

This research was supported in part by PHS Grant Nos. CA69043 and CA70209 by the National Cancer Institute. The authors would like to acknowledge Dr. Wei Yue and Dr. Jiping Wang of the University of Virginia for help in their experiments. They would also like to acknowledge Dr. Andrew Karellas of the University of Massachusetts Medical Center for helpful discussions.

[a]Author to whom correspondence should be addressed; electronic mail: h17y@virginia.edu

[1]W. R. Chen, R. L. Adams, S. Heaton, D. T. Dickey, K. E. Bartels, and R. E. Nordquist, ''Chromophore-enhanced laser-tumor tissue photothermal interaction using a 808 nm diode laser,'' Cancer Lett. (Shannon, Ireland) **88**, 15–19 (1995).

[2]W. R. Chen, R. L. Adams, K. E. Bartels, and R. E. Nordquist, ''Chromophore-enhanced in vivo tumor cell destruction using an 808-nm diode laser,'' Cancer Lett. (Shannon, Ireland) **94**, 125–131 (1995).

[3]W. R. Chen, R. L. Adams, A. K. Higgins, K. E. Bartels, and R. E. Nordquist, ''Photothermal effects on murine mammary tumors using indocyanine green and an 808-nm diode laser: An *in vivo* efficacy study,'' Cancer Lett. (Shannon, Ireland) **98**, 169–173 (1996).

[4]W. R. Chen, R. L. Adams, and R. E. Nordquist, ''Laser-photosensitizer assisted immunotherapy: A novel modality in cancer treatment,'' Cancer Lett. (Shannon, Ireland) **115**, 25–30 (1997).

[5]W. R. Chen, K. Robinson, R. L. Adams, A. K. Singhal, and R. E. Nordquist, ''Anti-tumor immune responses induced by the treatment of photodynamic immunotherapy,'' Proc. SPIE **3254**, 27–34 (1998).

[6]W. R. Chen, W. G. Zhu, J. R. Dynlacht, H. Liu, and R. E. Nordquist, ''Long-term tumor resistance induced by laser photoimmunotherapy,'' Int. J. Cancer **81**, 808–812, 1999.

[7]W. R. Chen, H. Liu, A. Nordquist, and R. E. Nordquist, ''Tumor cell damage and leukocyte infiltration after laser immunotherapy treatment,'' Laser Med. Sci. (in press, 1999).

[8]W. R. Chen, H. Liu, and R. E. Nordquist, ''Dynamically observing intratumor injection of laser-absorbing dye and immunoadjuvant using digital x-ray imaging technique,'' Cancer Research (submitted).

[9]H. Liu, L. L. Fajardo, and B. C. Penny, ''Signal-to-noise ratio and detective quantum efficiency analysis of optically coupled CCD mammography imaging systems,'' Acad. Radiol. **3**, 799–805 (1996).

[10]H. Liu, J. Xu, G. Halama, and J. McAdoo, ''Digital fluoroscopy using an optically coupled CCD: Preliminary investigations,'' Proc. SPIE **2976**, 256–261 (1997).

[11]J. H. Hubbell, ''Photon mass attenuation and energy-absorption coefficients from 1 keV to 20 MeV,'' Int. J. Appl. Radiat. Isot. **33**, 1269–1290 (1982).