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Radiation effects on physically cross-linked agarose hydrogels*

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As a potential matrix of three-dimensional gel dosimeter, agarose hydrogels will be used for measuring radiation doses, hence the importance of studying their radiation resistance and radiolysis mechanism. Physical property and chemical structure of physically cross-linked agarose hydrogel samples irradiated to 0–200 kGy by ^{60}Co γ-rays were analyzed by universal testing machine, gel permeation chromatography, fourier transform infrared spectrometer, ultraviolet visible spectroscopy, nuclear magnetic resonance, and gas chromatography. The results showed that agarose hydrogels had good radiation stability below 25 kGy, and the maximum compression strength of sample was *ca.* 0.1 MPa at 25 kGy. The irradiated samples degraded obviously and liquefied gradually with increasing doses. Compared with unirradiated sample, carbonyl groups, which generated from the molecular chains of agarose hydrogels, were observed at 25 kGy and increased gradually with dose. The main gas products evolved from irradiated agarose hydrogels were H_2, CO_2, CO and CH_4. Based on the analysis of radiolytic products, the radiolysis mechanism of agarose hydrogels under γ-radiation was proposed.

Keywords: Agarose, Hydrogel, γ-radiation, Radiolytic products, Structure analysis

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I. INTRODUCTION

In some radiation applications, three-dimensional dose distribution should be calibrated. The gel dosimeter is the only way to provide information of three-dimensional dose distribution [1], being crucial for dosimetry monitoring in a volume of special shape or a distinctive nuclear environment. Fricke gel dosimeter, with ferrous salt components dispersed in gel, is widely used in dosimetry determination, though it is difficult to have a well-distributed dispersion of ferrous ions in gel dosimeter, hence a large measurement error.

The working principle of acrylamide gel dosimeter is radiation polymerization of acrylamide monomer in solution, and the dose response is closely related to crosslinking degree. This is important in clinic treatments where high dose gradients exist such as intensity-modulated radiation therapy and stereotactic radiosurgery but larger measurement errors are caused by the oxygen effect and post-irradiation effect [1]. Therefore, the combination of polymer gels as both the carriers and radiochromic materials with small diffusion coefficient is a new direction of gel dosimeters.

As a linear natural polysaccharide, agarose has a basic structure of repeating units of alternating 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose. Dissolved in boiling water, agarose molecular chains form gels through hydrogen bond at about 35 °C. There exists helical structure in the agarose molecular chains determined by X-ray diffraction and optical rotation, and single chain spiral structure transforms into the double helix structure attributed to the action of hydrogen bond [2, 3]. Since its three-dimensional network structure, agarose hydrogel has certain mechanical strength and stable chemical properties. More importantly, agarose can form high strength and elastic gels without long time pretreatment for physical or chemical gelation process, which is necessary for acrylamide or polyvinyl alcohol hydrogels. As an excellent natural carrier material, agarose hydrogel dosimeters suffer from radiations in dosimetry monitoring, hence the changes in physicochemical properties, and the worsening dosimetry performance. So, it is necessary to investigate the radiation effect on agarose hydrogels. Clemens von Sonntag *et al.* [4, 5] did a lot of research on radiation chemistry of polymers, involving the preliminary reaction mechanism and kinetics in aqueous solution of neutral sugar after γ-irradiation, indicating that ·OH and other fragment radicals play a significant role in the radiolysis of polymers. Recently, it is reported that some water soluble natural polymer derivatives such as carboxymethyl chitosan (CMCts) [6], carboxymethyl cellulose (CMC) [7–9], and some nanocomposite [10], in viscous solution state (in concentrations of 10%–50%) could react to form hydrogels by means of radiation crosslinking. In this paper, we report γ-ray radiation effects on physicochemical properties of the physically cross-linked agarose hydrogels, including the gel phase, mechanical properties, molecular weight, chemical structure, etc.

II. EXPERIMENTAL

A. Materials

Agarose (LP0028A, M_W = 100 000 g/mol) was from British OXOID company and used without purification.

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Deionized water was prepared through the Purelab flex pure water equipment (ELGA, UK).

B. Preparation of agarose hydrogels

The agarose powder was mixed with deionized water to prepare 6wt.% agarose solution and refluxed to boiling point. Physically cross-linked agarose hydrogel was obtained after cooling to room temperature ((298 ± 3) K).

C. Irradiation and characterization of samples

1. *Irradiation of samples*

The agarose hydrogel was cut into $1\,\text{cm} \times 1\,\text{cm} \times 0.5\,\text{cm}$ samples. They were placed into sample tubes, which were evacuated by a glass vacuum system, filled with N_2 (99.99%) of up to 26.7 kPa, and sealed using oxyacetylene flame. The sealed tubes were irradiated to different doses (0–200 kGy) by ^{60}Co γ-rays at a dose rate of 168 Gy/min.

2. *The testing of mechanical properties*

The compression performance of irradiated samples was measured by CMT4104 universal material testing machine (SANS, USA) at room temperature. Sample size: $\Phi 25\,\text{mm} \times 5\,\text{mm}$; Compression sensor: 10 kN; Compression speed: 0.5 mm/min.

3. *Molecular weight analysis*

Agarose hydrogel samples dried by infrared lamp were dissolved separately by extraction solution to form a solution of 0.01wt.%. Molecular weight of the samples were measured with WH2-04/WTREX-02 GPC (Wyatt, USA), and the extraction solution was 0.1 mol/L NaCl/0.1 g/L NaN_3 aqueous solution. The column temperature was 308 K, and flow rate was 0.5 mL/min.

4. *FTIR analysis*

Agarose hydrogel samples were dried up to dehydration by infrared lamp. They were mixed with KBr in a agate mortar. The mixture was analyzed by an infrared spectrometer of Nicolet 6700 (Thermo Fisher, USA).

5. *UV-vis analysis*

Agarose hydrogel samples before and after irradiation were dissolved to 0.1wt.% solution, for measuring their absorbance on a Lambda 950 UV-vis spectrophotometer (Perkin Elmer, USA) at room temperature.

6. *NMR analysis*

The dried samples were diluted in deuterated water to 5 mg/mL and measured on a Bruker Avance 600 NMR (Bruker, Switzerland) at 600 MHz. The system's chemical shift scale had been calibrated to 0 ppm with tetramethylsilane.

7. *GC analysis*

An irradiated tube was connected to the gas sampling system. The quantitative analysis of gases evolved from irradiated samples was carried out through HP 6890 gas chromatography equipped with 5A molecular sieve column and PropakQ column (HP USA) by the method of external standard, at column temperature of 291 K. Thermal conductivity detector (TCD) was utilized to determine the signal of samples.

III. RESULTS AND DISCUSSION

A. Physical properties vs. absorbed dose

Diluted aqueous solution (below 10%) of polysaccharides such as cellulose, starch, chitin/chitosan and their water-soluble derivatives have been proved as degradable type polymers under ionizing radiation. It was reported that their viscosity decreased greatly with increasing doses [11]. In this study, to elucidate the irradiation effect on agarose hydrogel, physically cross-linked hydrogels were irradiated at different doses. The phase of irradiated agarose hydrogels was closely related to the absorbed dose. As shown in Fig. 1, the phase of samples changed slightly below 50 kGy, while the samples were partially liquefied at 100 kGy and their phases showed obvious changes at 150 kGy.

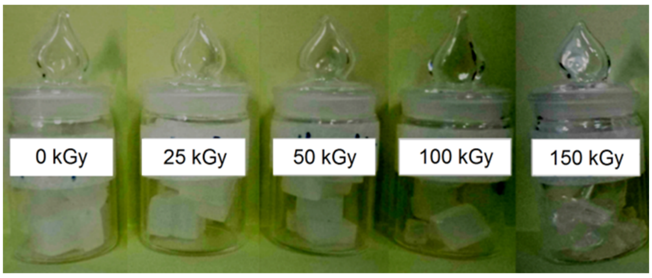

Fig. 1. (Color online) Phase changes of the agarose hydrogels irradiated to different doses.

To get an intuitive understanding of the changes in the physical properties, compression performance of agarose hydrogel before and after irradiation was analyzed (Fig. 2). The compression strength and strain of agarose hydrogels decreased with increasing dose, and so did the initial modulus. Similar phenomena were observed in carrageenan gel [12], and the sample strength decreased after high dose irradiation.

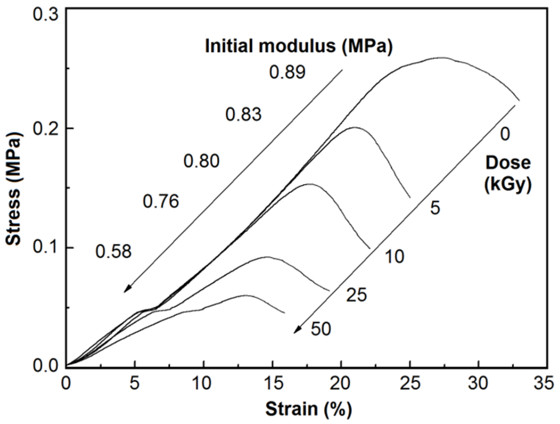

Fig. 2. The stress-strain curve of agarose hydrogels irradiated to different doses.

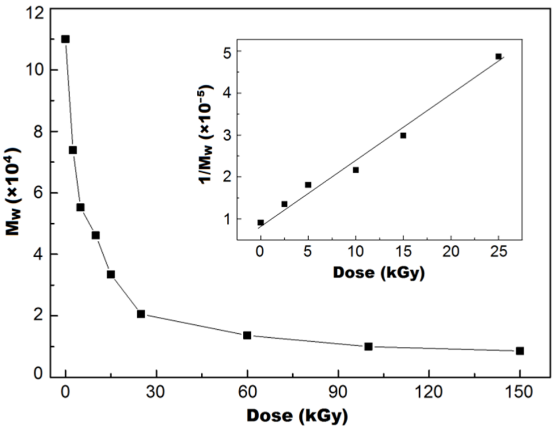

Fig. 3. Dose effect on molecular weight of agarose. The inset shows dosimetry response of $1/\mathrm{M_W}$ below 25 kGy.

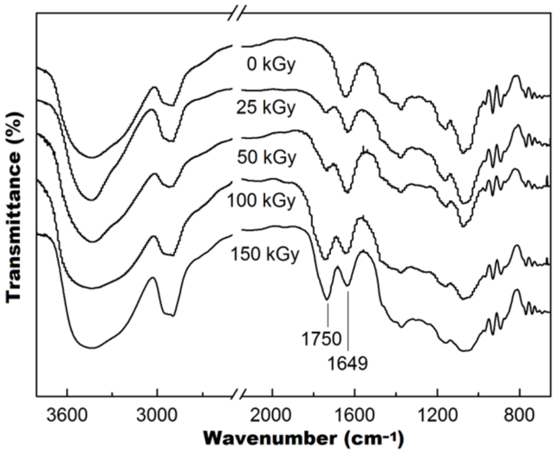

Fig. 4. FTIR spectra of agarose hydrogels irradiated to different doses.

Figure 2 indicates that the γ-rays destroyed three dimensional network structure of cross-linked agarose molecules in hydrogel, resulting in relaxation of the gel networks, with low gel rigidity and toughness. The yield point of samples appeared at about 0.05 MPa stress and about 5% strain, and it will no longer obey the elastic deformation regularity after the yield point. Therefore, physical properties of agarose hydrogel decrease with increasing dose, while the agarose hydrogel degrades under the γ-ray irradiation.

B. Molecular weight vs. absorbed dose

For evaluating degradation degree of agarose hydrogels, GPC was employed to measure molecular weight ($\mathrm{M_W}$) of irradiated agarose hydrogels. As seen in Fig. 3, $\mathrm{M_W}$ of the irradiated samples decreases rapidly with increasing doses up to 25 kGy, where it begins to drop slowly to 1×10^4 at 150 kGy. This shows that radiation degradation of agar hydrogel is dominant in early stages of the γ-ray irradiation. Degradation of polymer molecules with long chain obeys random degradation kinetics [13], i.e. $1/\mathrm{M_W} = A + kD$, where k is the rate of radiation degradation, D is absorbed dose and A is a constant related to degradation of polymer molecules. The insert of Fig. 3 shows that radiation degradation of agarose is consistent with the random degradation kinetics at 0–25 kGy, and the radiation degradation rate is $k = 1.52 \times 10^{-6}$ mol/J.

C. Chemical structure vs absorbed dose

Radiation effects on chemical structure of agarose hydrogel were studied by FTIR, UV-Vis, NMR and GC.

FTIR spectra of agarose hydrogels before and after radiation are shown in Fig. 4. The characteristic peaks of agarose hydrogels are: $3400\,\mathrm{cm^{-1}}$, stretching vibration absorption peak of O—H; $1078\,\mathrm{cm^{-1}}$, the glucosidic bond; 1170 and $931\,\mathrm{cm^{-1}}$, C—O—C bridge bond in L-galactose furanose dehydrated at 3,6-position [14]; $1390\,\mathrm{cm^{-1}}$, bending vibration absorption peak of O—H; $2920\,\mathrm{cm^{-1}}$, vibration absorption peak of C—H, and $1649\,\mathrm{cm^{-1}}$, bending vibration peak of absorbed water [15]. Agarose hydrogel is decomposed gradually during irradiation, and the degree of degradation increases with dose, but the basic structure of agarose hydrogel still remains [16], so the location and intensity of the characteristic peaks does not change significantly. However, a new absorption peak at $1750\,\mathrm{cm^{-1}}$ occurs gradually as the dose increases. It is speculated to be characteristic absorption peak of carbonyl. UV-Vis spectra of the agarose hydrogel (Fig. 5) show that the carbonyl absorption peak of irradiated samples appears at 268 nm [17, 18], and its intensity increases with dose. The carbonyl absorption peak was observed at 265 nm with irradiated chitosan or carrageenan polysaccharide solution [6, 12]. Therefore, compound with carbonyl groups is a main radiolytic product of agarose hydrogel.

^{1}H NMR spectra of samples before and after irradiation are shown in Fig. 6. Due to the large molecular weight of agarose hydrogels, ^{1}H NMR spectrum cannot give the chemical structure in detail. However, a new aldehyde peak appears at 8.45 ppm, which is in accordance with the results of FTIR and UV-Vis. Zhang *et al.* [19] studied the radiation degrada-

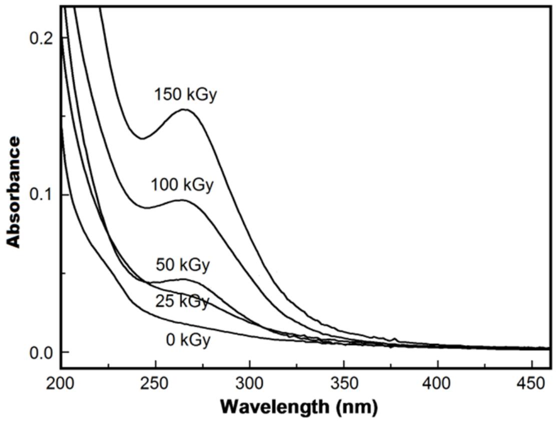

Fig. 5. UV-Vis spectra of agarose samples irradiated to different doses.

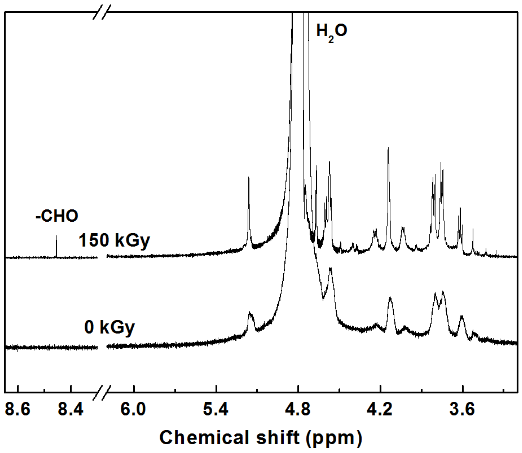

Fig. 6. ^{1}H NMR spectra of agarose samples irradiated to different doses.

tion of chitosan in aqueous solution and found that the radiolysis of chitosan was induced by ·H and OH· free radicals derived from radiolysis of water. Thus it can be speculated that the radiation degradation of agarose with similar structure is also induced by ·H and OH· free radicals, which leads to the cleavage of –C-O-C– bond in agarose molecules and the formation of carbonyl groups.

The analysis of gas radiolytic products from irradiated samples is important for the application of polymeric materials and proposing the radiolysis mechanism. As seen in Fig. 7, H_2 is the main gas radiolytic product which is partly due to the combination of ·H and ·H from water radiolysis. CO_2, in fewer contents, increases with doses, indicating that internal reactions in agarose hydrogel are intensified at high doses. Trace contents of CO and CH_4 were detected. G values of the gaseous products are given in Fig. 7. They are in the order of $G(H_2) > G(CO_2) > G(CO) > G(CH_4)$. These results indicate that the cleavage of C−H bond is the main radiolysis path, and the degradation of the C−C bond also appears during irradiation.

From the FTIR, UV-Vis, NMR and GC analyses, radiation degradation of agarose hydrogel shows similar radiolysis behavior to that of alcohol or ether [20]. The main radiation degradation process is as follows:

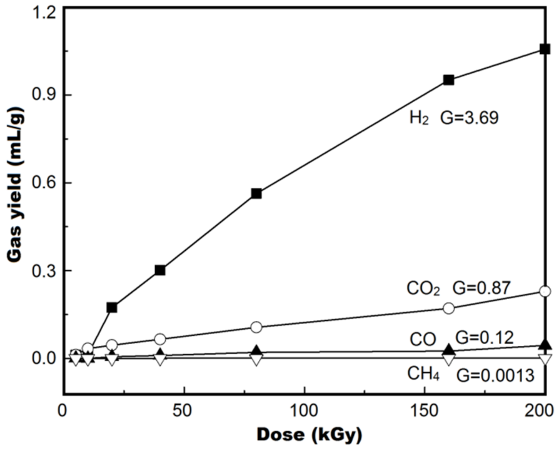

Fig. 7. Gas yield of agarose hydrogels irradiated to different doses.

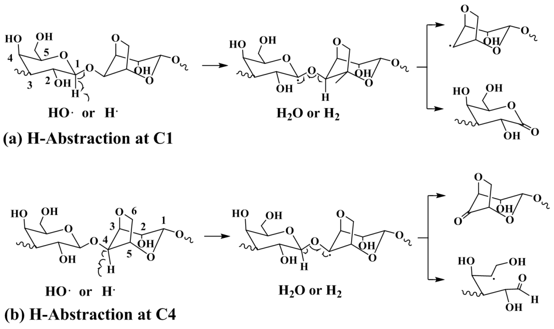

Fig. 8. The main radiolysis mechanism of agarose hydrogels. (a) ·H or ·OH abstraction at C1; (b) ·H or ·OH abstraction at C4.

1) Hydrogen abstraction reaction: ·H or ·OH free radicals formed in water radiolysis attack agarose molecules, leading to H-abstraction reaction at all the carbons, and generating H_2/H_2O and agarose radicals;

2) Cleavage of glycosidic bond and formation of carbonyl: When the ·H or ·OH free radicals attack the molecules at the C1 position in β-D-galactopyranose and 3,6-anhydro-α-L-galactopyranose, or C3 in β-D-galactopyranose and C4 in 3,6-anhydro-α-L-galactopyranose. The cleavage of –C-O-C– in the main chain of agarose hydrogels leads to the formation of carbonyl group and aldehyde group (Fig. 8);

3) Scission reaction on main chain: Breaking C−C bond in the main chain of agarose hydrogels is extremely difficult due to its high bond energy, hence the formation of trace methane.

IV. CONCLUSION

The radiation effect of γ-rays on physically cross-linked agarose hydrogels was studied systematically by a variety of analyzing methods. The phase of agarose hydrogel is unchanged at absorption doses below 25 kGy and their compression property still reaches 0.1 MPa, indicating that agarose hydrogel keeps good radiation resistance as a matrix for dosimeter. Further analysis shows that products containing carbonyl are formed in the irradiated agarose hydrogels, releasing some gases such as H_2, CO_2 and CH_4. According to the analysis of radiolytic products, it is proposed that hydrogen abstraction reaction occurs at all the positions of carbons in agarose molecules attacked by ·H or ·OH free radicals. And the cleavage of ether bond in the main chain of agarose hydrogel leads to the formation of carbonyl group and aldehyde group.

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