1.2 Some Progress on Radiation Chemistry of Substances of Biological Interests and Biological Applications of Radiation Technology in China

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Abstract Studies in China on the detection method of irradiated food, mechanism of DNA damage induced by peroxidation, radiolysis of natural products and herbs are reviewed on the update open literature, and some progress on applications of radiation technology is summerized.

In China, more attention has been paid to the Radiation Chemistry research of substances of biological interest and natural products and applications of radiation technology in biomedicine. From the invited talks and contributed papers of Chinese at this symposium, one can get an overview of the recent achievements in radiation research and radiation processing. Here, we would only report some progress in China of our interests.

I. RADIATION CHEMISTRY OF SUBSTANCES OF BIOLOGICAL AND BIOMEDICAL INTEREST

The formation of peroxide from fatty part of pork under γ irradiation

Recent research results reveal that DNA damage induced by lipid-peroxidation may be involved in processes of ageing and tumorigenisis. Interaction of lipid peroxidation products with DNA was studied [1,2]. In the linoleic acid-DNA system, a Schiff base product with fluorescence was formed by the reaction of DNA with peroxidized linoleic acid and it increased with increasing of reaction time. DNA damage induced by liposome peroxidation was also reported. Among the four typical bases, the damage of guanine was much more serious than those of the other three.

The study of peroxide formation from fat and lipid under γ irradiation is important not only for the detection of irradiated food but also for the control of the food quality. It was discovered that the organic peroxide thus formed was proportional to the dose applied. (Fig. 1) [3]. Moreover, the peroxide content of irradiated pork during storage increased much more rapidly than that of unirradiated reference (Fig. 2) These results indicate that for the purpose of preservation (extension of the shelf-life), the irradiation technology applying to sausage seems doubtful.

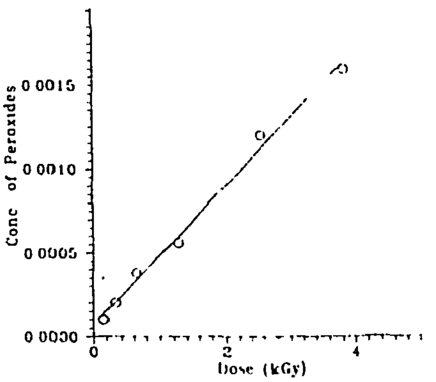

Fig. 1 The dependence of the formation of organic peroxides on absorbed dose of the irradiated pork.

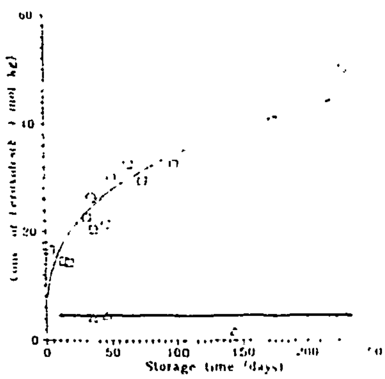

Fig. 2 The difference in the formation rate of peroxide between irradiated peroxidation and autooxidation at -18 °C. unirradiated (Δ) and irradiated (□, 2.18 kGy; ○, 3.12 kGy) pork

Study on the excitation of phosphoryl group ($\equiv P=O$) by photolysis, γ and pulse radiolysis of model compound TBP [5]

$\equiv P=O$ is an important functional group of DNA. The extinction coefficient of its $P=O$ bond is very low, thus it is very difficult to study the chemistry of the excitation state of this $P=O$. Yet, we have successfully detected TBP* molecule and measured its G value by different methods. Fig.3 and Fig.4 show the results obtained by pulse radiolysis, in which ketyl radical is formed *via* energy transfer from TBP*.

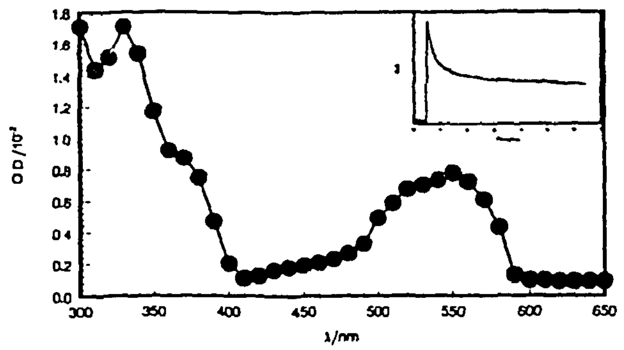

Fig. 3 Transient spectrum of N_2 degassed benzophenone (0.04 mol dm^{-3}) in TBP, 20μs after pulse. Insert, decay of absorbance at 550 nm, 5 Gy/pulse.

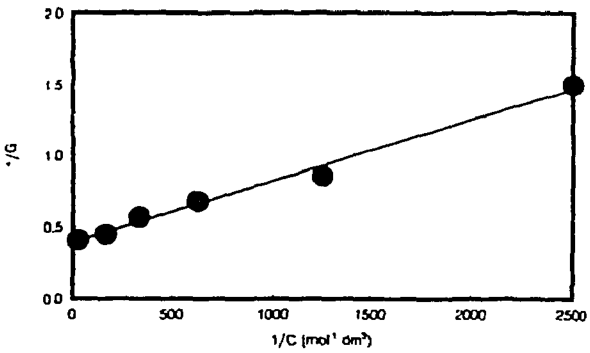

Fig. 4 Dependence of G(ketyl radical) on the concentration of Benzophenone in TBP. Degassed by N_2, 5 Gy / pulse.

The competition kinetics was also examined employing the following reactions and Tab. 1 complied the ratios of the rate k_2 to k_1 obtained from different systems by different methods.

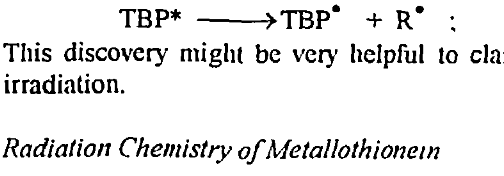

Table 1. Comparison of $*k_2/k_1$ and estimated τ of singlet of TBP obtained by different methods.

Method	k_2/k_1	$\tau_{singlet}$ / s
Photolysis of Φ_2SO-TBP	240	1.2×10^{-7}
γ radiolysis of Φ_2SO-TBP	250	1.2×10^{-7}
Pulse radiolysis of Φ_2CO-TBP	840	4.2×10^{-7}

* Assuming the second reaction is diffusion controlled with $k_2 = 2.0\times 10^9$ dm^3 mol^{-1} s^{-1}

The above mentioned results indicate that the radiation decomposition of TBP might be mainly due to the excitation of phosphoryl group, *i.e.* the following reactions may occur:

$$TBP^* \longrightarrow TBP^\bullet + R^\bullet \quad ; \qquad TBP^* \longrightarrow \text{Acid products}$$

This discovery might be very helpful to clarify the mechanism of the damage of DNA by UV and γ irradiation.

Radiation Chemistry of Metallothionein

Metallothionein (MT), a special protein containing 20 thiol groups, has high potential application as a medicine. The rate constants (Table 2)of MT reacting with various active species from radiolysis of water have been studied by pulse radiolysis and γ radiolysis.

The pulse radiolysis of metallothionein shows the formation of disulphide anion [6,7].

$$RS^\bullet + RS^- \rightleftarrows RSSR^{\bullet -}$$

Fig. 5 shows that the $RSSR^{\bullet -}$ radical anion was formed by intermolecular reaction, however, final radiolytic products analysis indicated that the disulphide linkage was mainly formed intramolecularly [8]

Moreover, a very interesting phenomenon was discovered that the modified MT containing disulphide can be strongly adsorbed by PO_4^{3-} of DNA (Fig. 7).

Table 2. Compilation of the rate constants of some reactions involved in the radiolysis of metallothionein (RS^-) and cysteine (CyS^-)

Reaction	Rate constant / $dm^3\ mol^{-1}\ s^{-1}$	
	RS^-	Cys^-
$OH + RS^- \longrightarrow RS^\bullet$	6×10^{10}	3.4×10^{10}
$RS^\bullet + RS^- \longrightarrow RSSR^{\bullet -}$	1.8×10^9	1.9×10^9
$*RSSR^{\bullet -} \longrightarrow RS^- + RS^\bullet$	7×10^4	3.2×10^5
$RS + RSSR^{\bullet -} \longrightarrow RSSR + RS^-$	9.2×10^8	4×10^9
$RSSR^{\bullet -} + O_2 \longrightarrow RSSR + O_2^{\bullet -}$	3×10^7	4.3×10^8

* The units of rate constant for this reaction are s^{-1}.

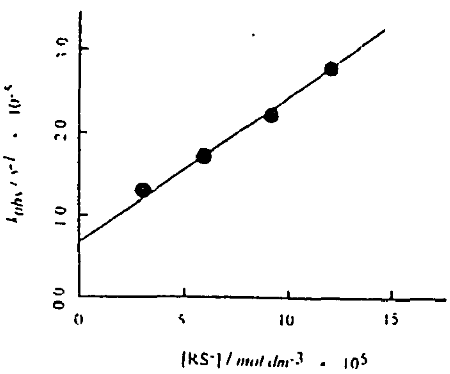

Fig. 5 The build-up kinetics of $RSSR^{\bullet -}$ showing its dependence on RS^- concentration. Bubbled with N_2O, 1 Gy/pulse, monitored at 450 nm.

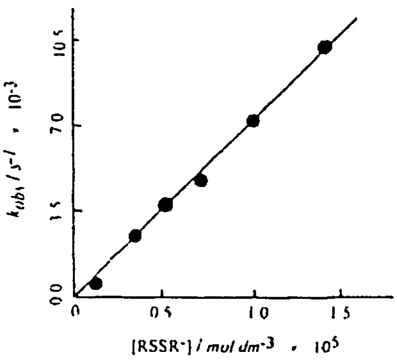

Fig. 6 The decay kir ;tics of $RSSR^{\bullet -}$ monitored at 450 nm. Solution bubb. 'd with N_2O (O_2 free).

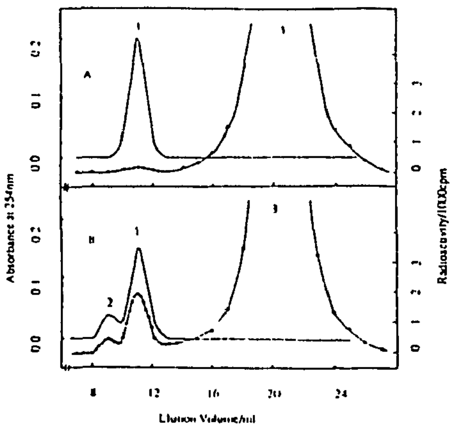

Fig .7 Gel-filtration Chromatography profiles of mixtures of sodium [^{32}P]phosphate with (A) native and (B) pre-irradiated MT after 24-h incubation at 25 °C, performed on a Sephadex G-50 column eluated by 0.01 mol dm^{-3} Tris HCl containing 0.1 mol dm^{-3} phosphate buffer (pH7.0). Peak 1 and 2, monomeric and dimeric forms of MT; peak 3, free [^{32}P]phosphate. (—) absorbance at 254 nm, (-o-) [^{32}P]phosphate radioactivity.

Radiation Chemistry of medicine

(1) Using ethanol as a solvent probe to detect the active site of baicalin [10]

The results show that the active sites of baicalin are at its π conjugated system containing carbonyl group (ring B and C) and the capability of scavenging electron by carbonyl groups is evidently lower than that of acetophenone. The conjugated system of ring B and C can capture OH, RO$^{\bullet}$, H$^{\bullet}$ and $RO_2^{\bullet}$ radicals effectively. It is helpful to explain the pharmacological effect of flavonoid.

(2) Studying the effect of water on the radiation decomposition of the chief components of herbs [11]

Table 3. The variation of bioactive components with H_2O contents in γ-irradiated herbs.
Absorbed dose: rhubarb 150 kGy, other herbs 10 kGy.

Herb		content of components (%)			
Scutellaria	H_2O	0	15	30	45
Baicalensis	Baicalin	13.78	13.25	12.98	12.71
Glycyrrhiza	H_2O	0	19	30	40
Uralensis	Glycyrrhizic Acid	1.6	1.24	1.08	1.06
Radix Gentianae	H_2O	0	17.3	29.2	39.4
Marcrophyllae	Gentiopicrin	4.16	3.34	3.24	3.36
rhubarb	H_2O	0	15	23	35
	Anthraquinone	2.13	1.94	2.01	1.86

Table 3 reveals that water is able to promote the γ radiolysis of herbs, but it seems that water takes an effort on the surface of herbs. a detailed study has been undertaken on rhubarb-H_2O system.
The additives such as fructose and glucose can inhibit the γ radiation decomposition of herbs.

(3) Determination of one electron reduction potentials of some radiosensitive compounds by pulse radiolysis [12]

Anthraquinone-2-sulphate (AQS), duroquinone (DQ) and methyl viologen (MV^{2+}) were used as references. The results were shown in Table 4.

Table 4. Rate constants of electron transfer and equilibrium constants for reaction
$$D^{\bullet -} + A \underset{k_-}{\overset{k_+}{\rightleftharpoons}} D + A^{\bullet -}$$
and calculated values of their one-electron reduction potentials

D	A	k_+ / $dm^3\,mol^{-1}\,s^{-1}$	k_- / $dm^3\,mol^{-1}\,s^{-1}$	K	ΔE /mV	$E(D/D^{\bullet -})$/mV
Miso	MV^{2+}	1.92×10^{8}	1.70×10^{9}	0.113	-56.0	-391, +3
911	AQS	1.15×10^{9}	1.28×10^{8}	8.98	56.4	-431, +2
CMNa	DQ	6.71×10^{8}	2.09×10[illegible]	32.1	88.9	-464, +2
SMU-1	AQS	6.06×10^{9}	7.43×10^{7}	81.5	113	-488, +2
SMU-2	AQS	6.99×10^{9}	3.90×10[illegible]	179.1	133	-508, +2
S3	AQS	6.96×10^{9}	7.02×10^{7}	99.1	[illegible]	-493, +2
SMD	AQS	2.58×10^{9}	6.69×10^{7}	38.6	[illegible]	-469, +2
SNN	AQS	3.54×10^{9}	3.05×10^{8}	11.6	62.7	-438, +2
BSO	AQS	4.69×10^{9}	1.01×10^{8}	46.4	98.3	-473, +2

II. RESEARCH ON BIOMEDICAL APPLICATIONS OF RADIATION TECHNOLOGY

Since 1980, especially late 80's, the radiation technology for biomedical application in China have been rapidly developed and its bright future is attracting a great of attentions.

Progress on γ-sterilization of biomedical products

(1) Evaluation of irradiated herbs [11]

Evaluation of pharmacological efficacy after γ radiation was performed on 124 types of herbs and fruitful results were obtained, which shows a hopeful prospect to sterilizing herbs by γ rays, yet important rules as following step should be obeyed:
a. Herb to be irradiated must exist in dry state
b. It is encouraged to make bolus by mixing powered Chinese medicine with honey to inhibit the radiation deconstruction of herbs.
c. The producer must strictly control the manufacture procedure to minimize the microbiological contamination, which subsequently lowers the dose applied.

(2) Improvements on hygienic level of glucoamylase[13]

γ-sterilization of industrial glucoamylase under 2 kGy can greatly reduce the contamination of gemma bacillus and other putrefactive microbes and thus stabilize the pH value during preparation Glucoamylase thus treated can be preserved at good quality even without antiseptics

(3) New treatment for enhancing the radiation tolerance of polypropylene .

Serial studies of radiation effects on PP have been carried out, such as the effect on the morphology of polypropylene in the presence of some additives [14]. To limit ETO method for sterilization, the new treatment of PP must be cheaper and safer than this conventional treatment. A lot of trails have been performed. Some treated PP present good radiation tolerance in the absence of additive Trinuvin 770, a well-known photo-oxidant stabilizer which might have blocking effect on autonomic nerve.

Progress on the Use of radiation processing for synthesis of biomaterials

Many studies focused on immobilization, synthesis of hydrogel, grafting hydrophilic monomer to biocompatiable polymer matrix and fundamental studies.

(1) Immobilization of Drugs, Enzymes and Cells

Slow release anti-cancer drugs(5-Fluorouracilum, Mitomycinum, Bleomycinum and Ara-C) were prepared by radiation co-polymerization of monomer such as HEMA. The release rate and amount of drugs can be controlled by regulating the content of hydrophilic or hydrophobic monomer(MMA<EMA<BMA) and crosslinking reagent in drug-copolymer composite and by changing the radiation dose and drug content. Pore structure of the composite may accelerate the release rate[15,16]. Radiation polymerization was used to immobilize testosterone propionate into crosslinked network of polyhydroxyethyl methacrylate to prepare slow release male sex hormone drug which is applied for testicular prosthesis[17].

The immobilization of enzymes (horseradish peroxidase (HRP), glucose oxidase), cells (baker's yeast, E. Coli), proteins (bovine serum albumin (BSA)) by radiation polymerization was also studied The monomers used were 2-hydroxyethyl methacrylate, acrylic acid, acrylamide and N-2-propyl acrylamide [18-21].

Generally, two methods have been applied for immobilization One method is radiation polymerization. Addition of crosslinking agent will significantly low the dose applied for gel formation and modification other properties. The other was graft co-polymerization, for example, the acrylic acid was grafted *via* radiation onto segmented polyetherurethane (SPEU) film, a kind of biocompatible material, and the peroxidase was immobilized onto the grafted SPEU film by chemical binding [22].

Immobilization of cell membrane of E. Coli by radiation technology was successfully performed. Nylon 6 was grafted with HEMA, which had "live" free radicals. The matrix containing free radicals was used to immobilize cell membrance of E. Coli by radiation entrapment at low temperature [20].

It should be pointed out that a number of irradiated oxidoreductase (catalase and HRP) are facile to be of post deactivated, especially in the air [23].

(2) Hydrogels

Recently, a lot of attentions have been paid to radiation synthesis of thermo-sensitive polymers and the hydrogel dressing. An activated copolymer, poly(N-isopropylacrylamide-co-N-acryloxy succinimide) (NASI) has been prepared. The active ester group of NASI has high chemical stability and good physical properties and shows both high reactivity and selectivity toward amino nucleophiles, thus, the BSA and HRP have been immobilized onto the active copolymer. The BSA (or HRP) / copolymer conjugates still maintain the original thermo-sensitive properties of the linear poly NIPAAm [24]. Other reports revealed that NIPAAm was grafted onto surface of ethyl-vinyl acetate (EVA slice) by means of pre-irradiation grafting due to poly-NIPAAm's weak mechanical strength, and the grafted surface of EVA slice expresses higher mechanical strength than and similar thermosensitivity to poly-NIPAAm gel. The response to the change of temperature through the lower critical solution temperature (LCST) of the graft is more swift than that of the gel. The LCST of the grafted surface shifts to higher and the drop of swelling around the LCST decreases with increasing hydrophilicity of the graft when co-grafting NIPAAM with acrylic acid or acrylamide [25].

A radiation prepared hydrogel base wound dressing carrying special drug is developed for hospital applications. Main chemical materials used are polymers (*e.g.* PVA, PVP, PEG), difunctional monomers (*e.g.* itaconic diallyl ester) and chloramphenicolum. The dressing is possessed of high water absorbability. Standard tests for biomedical material were made and clinical results obtained proved its practice [26].

Besides the progress mentioned above, "radiation chemistry and environment" has attracted more attentions recently. In order to reduce chemical fumigation, a new ^{60}Co facility (500 kCi) has been set up in Beijing for irradiation of rice to kill insects. A plan to promote the use of γ sterilization to substitute ethylene oxide sterilization has been made, and a plan of establishing pilot plant to treat fuel gas by electron beam irradiation is undergoing.

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