From Sticky Mucus to Probing our Past: Aspects and problems of the Biotechnological use of Macromolecules

Datum/Zeit	Veranstaltungsort	Thema
Mi, 30.06.2010 12.15-13.45	SR 309 Carl-Zeiss-Str. 3	***Macromolecules as BioPharma mucoadhesives***
Do, 01.07.2010 08.15-09.45	SR 308 Carl-Zeiss-Str. 3	***Macromolecules as vaccines***
Do, 01.07.2010 13.15-14.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing I. Thermal Processing, D, z and F values***
Fr, 02.07.2010 08.15-09.45	HS Haus 1 August-Bebel-Str. 2	***Stability in response to Bioprocessing II: Irradiation and freezing***
Fr, 02.07.2010 12.15-13.45	SR 307 Carl-Zeiss-Str. 3	***The use of non-recombining parts of the Y-chromosomal DNA and mitochondrial DNA as a probe into our past***

Stability in Response to Bioprocessing II: Irradiation and Freezing

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Overview: Principal methods for food sterilization

Heating

- **Thermal processing (canning)**
- **Microwaving**
- **Ohmic heating**

Chemical treatment

- **e.g. salting, pickling…**

Irradiation (ionizing radiation)

- **Food treated with high energy electromagnetic radiation, usually from a radioisotope source**
- **Dependent upon dose, can sterilize, reduce microbial population, kill parasites or insects, inhibit sprouting or germination…..etc.**

Why irradiate food ?

- to minimise food loss
- extend shelf life
- prevent contamination

- WHO estimates of storage losses
  - Cereal grains and legumes to be more than 10 %
  - Non-grain staples, vegetable and fruits through microbial contamination / spoilage ~ 50 %

- High proportion of raw foods considered infected: ~ 1 in 7000 eggs could contain Salmonella

Historical perspective

1896 **Wilhelm Röntgen discovers X-rays, produced when electrons brought to rest by matter (awarded first Nobel prize for physics in 1901)**

1921 **Schwartz uses X-Rays to kill parasites in meat**

1930 **German Otto Wüst issued a French patent for the preservation of foods by irradiation**

1940s **readily available ^{60}Co and ^{137}Cs suggested use for food irradiation**

1959 **First commercial food (spices) irradiation facility was commissioned in Federal Republic of Germany**

1976 WHO/FAO/IAEA guideline gave a clean bill of health to several irradiated foods. Recommended food irradiation be classified as a physical process

1980 WHO/FAO/IAEA : *' irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxological hazards; hence toxicological testing of foods so treated is no longer required'.*

1992 Irradiated foods allowed in UK

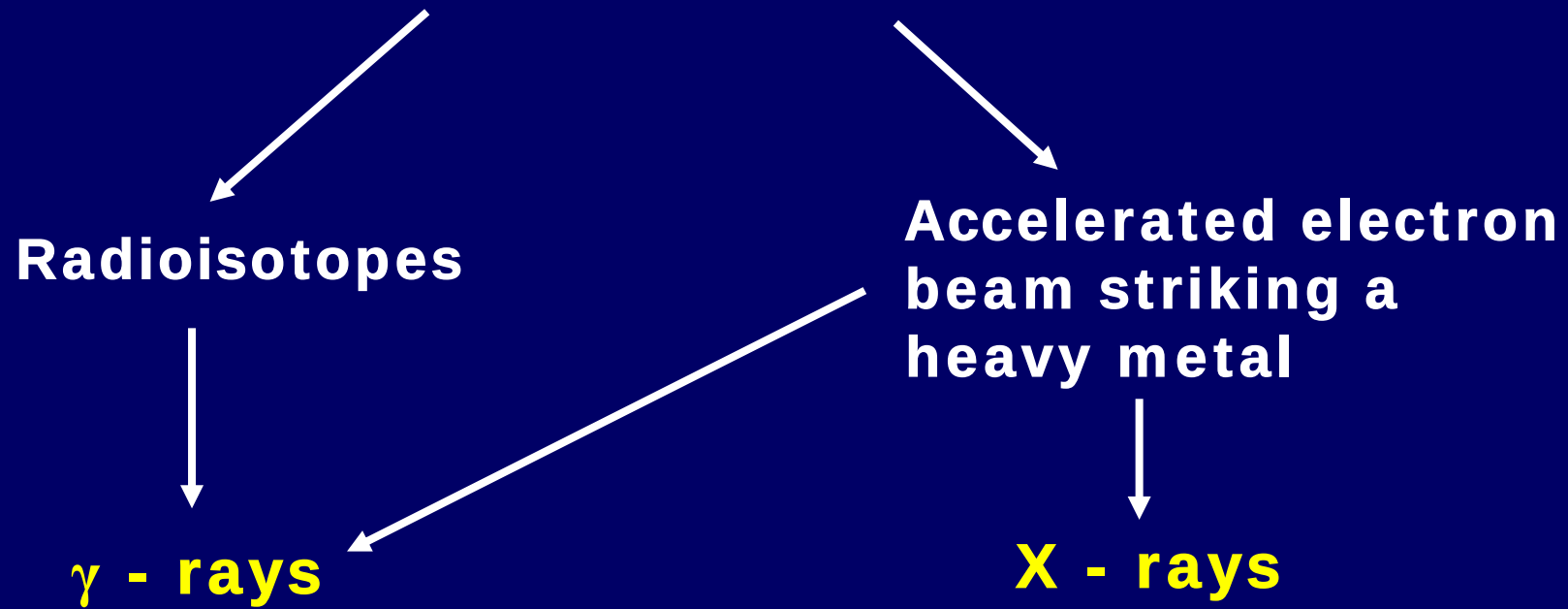
1996 40 countries have legal clearance for irradiation of one or more foods; 28 countries apply food irradiation commercially.

1997 Joint FAO/IAEA/WHO study group on High Dose food irradiation declared that foods irradiated at any dose are safe and that there is no need for upper dose limits

1 Gy (Gray) = 1 J of energy absorbed by 1 kg of matter

Product	Purpose of Irradiation	Dose permitted (k Gy)
FAO / IAEA / WHO Expert committee 1976		
Potatoes Onions	Sprout Inhibition	0.03-0.15
Wheat Ground wheat prod. Rice	Insect disinfection	0.1-1
Chicken Fish	Shelf-life extension / decontamination	2-7
FAO / IAEA / WHO Expert committee 1980		
Any food product	Sprout inhibition shelf-life extension / decontamination Insect disinfection control of ripening growth inhibition	Up to 10

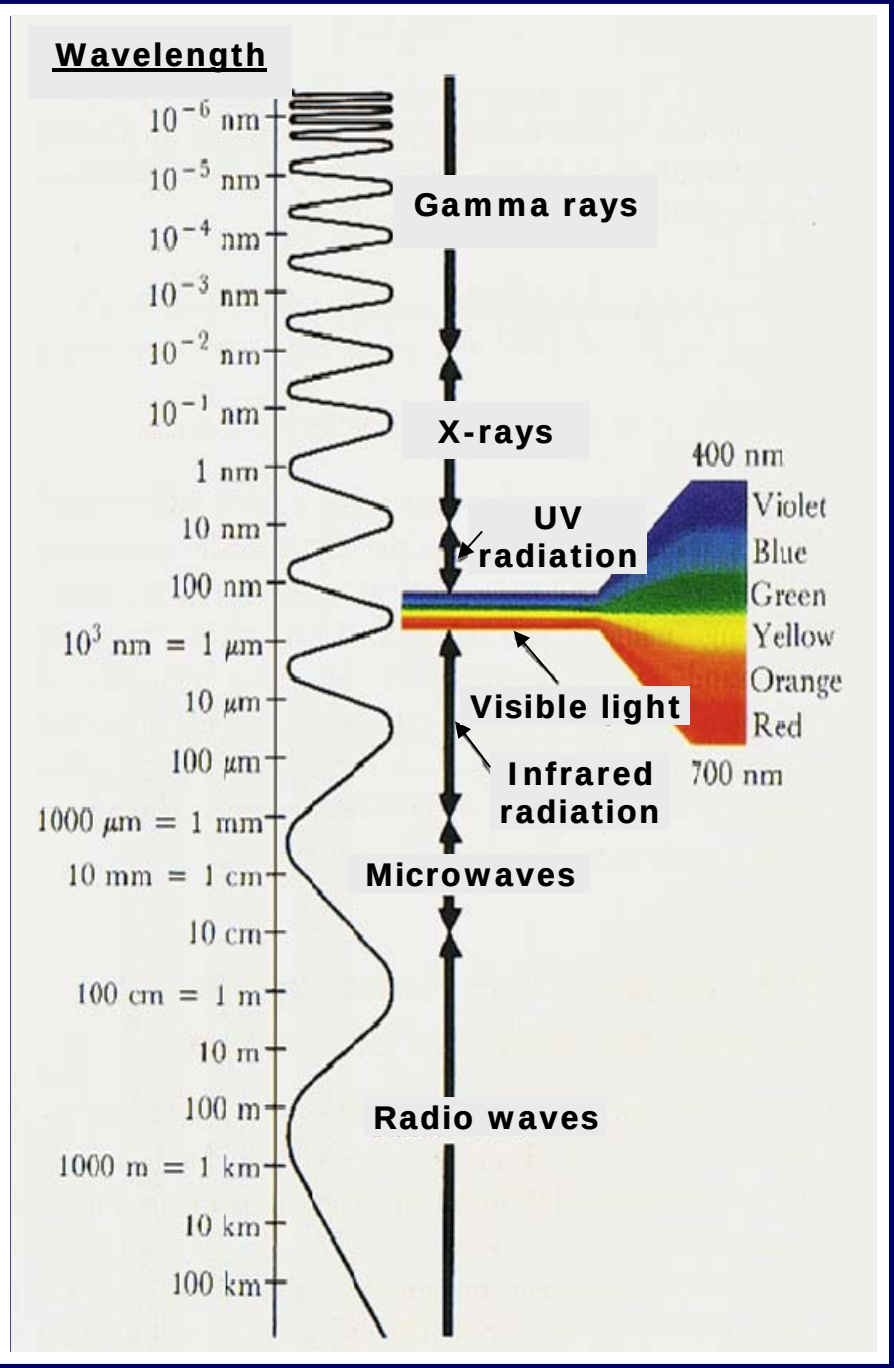
Sources of ionizing radiation



^{60}Co : Comonly used, low cost, available by-product of nuclear power reactors. Pellets (1 mm x 1 mm) or rods (1.84 mm x 25.4 mm)

^{137}Cs: less available, results from the fission of Uranium

Synchrotron: high intensity. Very high cost. Produces X- and γ-rays. No radioactive waste from machine sources



The electromagnetic spectrum

Energy of electromagnetic radiation:

$$E = h f$$

E = quantum energy

h = Planck's constant;

f = frequency (Hz)

1 eV = 1.6 x 10^{-19} J

Production and decay of ^{60}Co

Produced by bombarding Cobalt with neutrons:

$$^{59}_{27}Co + {}^{1}_{0}n \rightarrow {}^{60}_{27}Co + \gamma$$

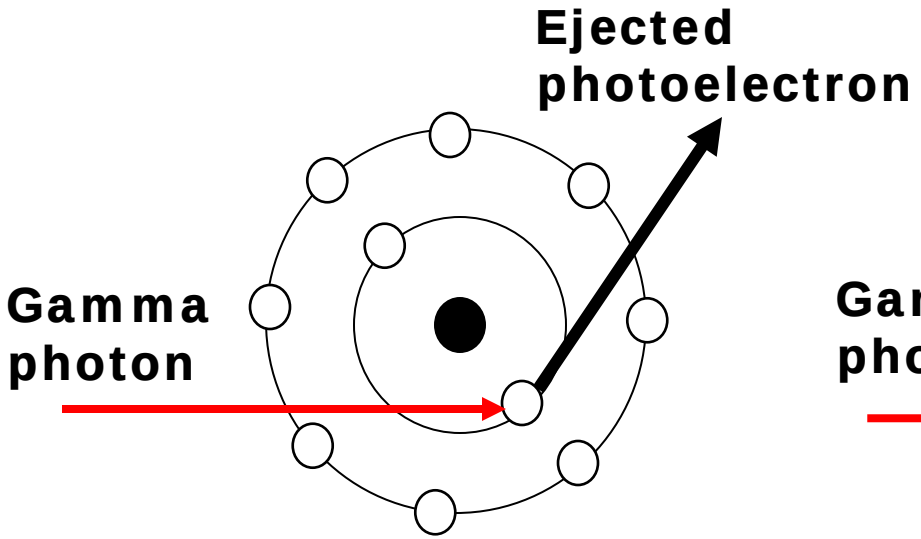
Decay produces gamma radiation at two wavelengths (energies):

$$^{60}_{27}\mathrm{Co} \rightarrow \begin{cases} \rightarrow \beta^{-} + \tilde{\upsilon} + E_{\beta max}\ (0.31\,\mathrm{MeV}) \\ {}^{60}_{28}\mathrm{Ni} \rightarrow {}^{60}_{28}\mathrm{Ni} + \gamma + \gamma \end{cases}$$

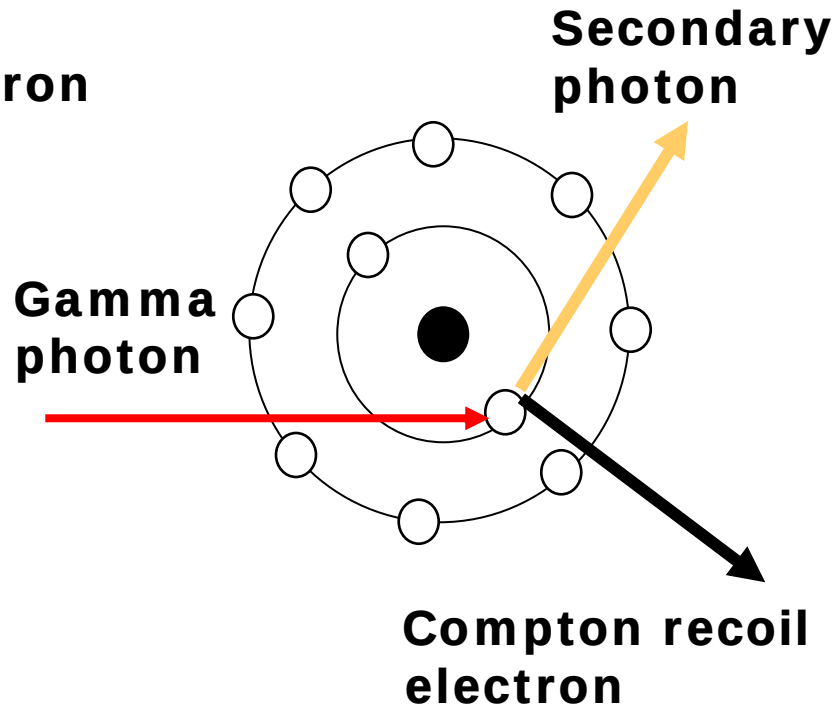
$t_{1/2}$ = 5.26 years *excited* *de-excited* **1.17 MeV** **1.33 MeV**

Interaction of ionizing radiation with matter

The photoelectric effect (< 60 keV)

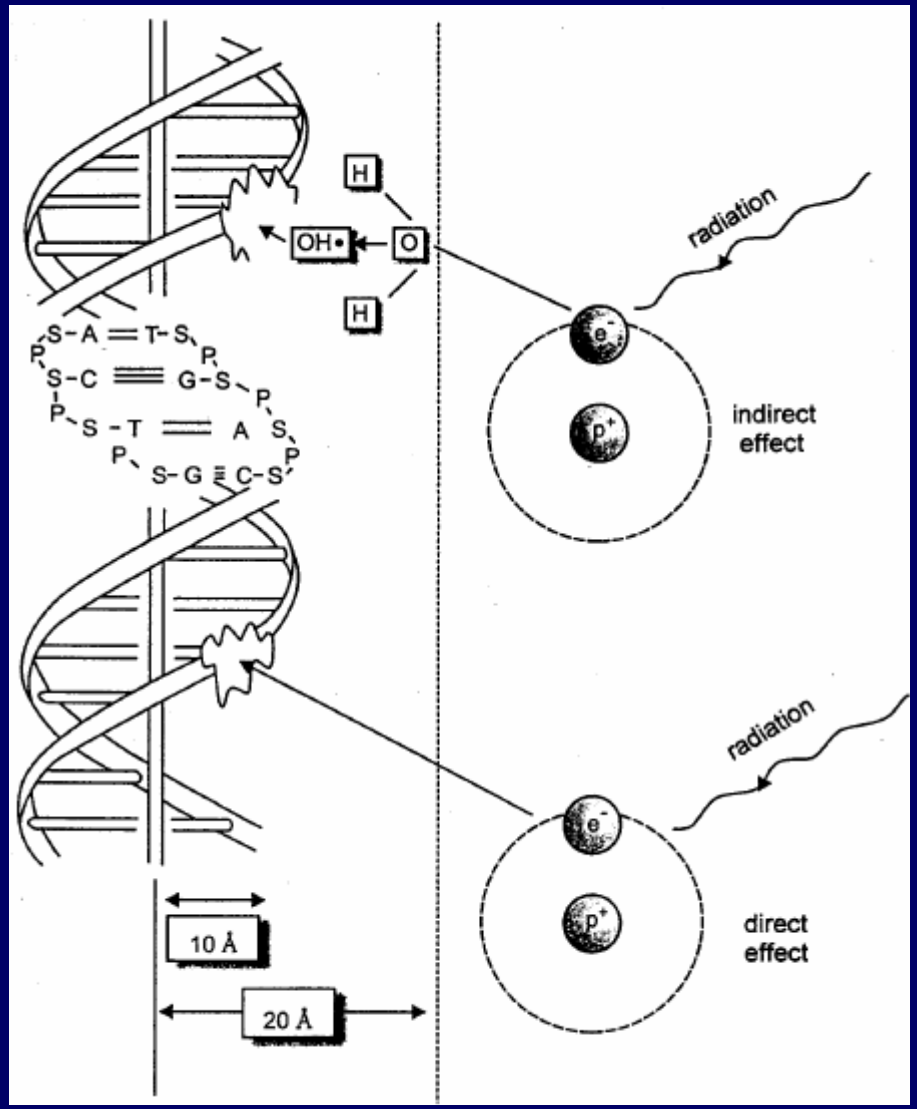


The Compton effect (>1 MeV)



Gamma radiation used in food irradiation has sufficient energy for the Compton effect (photon scattered off electron at longer wavelength)

Interaction of ionizing radiation with DNA



Irradiation in food processing

"Old" dose definition

Radurization

Radicidation (10-20 kGy)

Radappertisation (35-50 kGy)

Low dose (< 1 kGy)

- Inhibition of sprouting, germination
- Control of ripening
- Killing insects in cereal grains, fruits, etc.

Medium dose (1- 10 kGy)

- Killing food poisoning bacteria such as *Salmonella* and *Campylobacter*
- Killing parasites such as *Trichinella spiralis* and *Taenia saginata* in raw meat
- Reducing microbial population => extension of product life (e.g. fresh fish, strawberries)
- Sterilization of packaging material

High dose (> 10 kGy)

- Sterilizing of food (e.g. meat, poultry)
- Reduction of bacteria contamination
- Enzyme inactivation

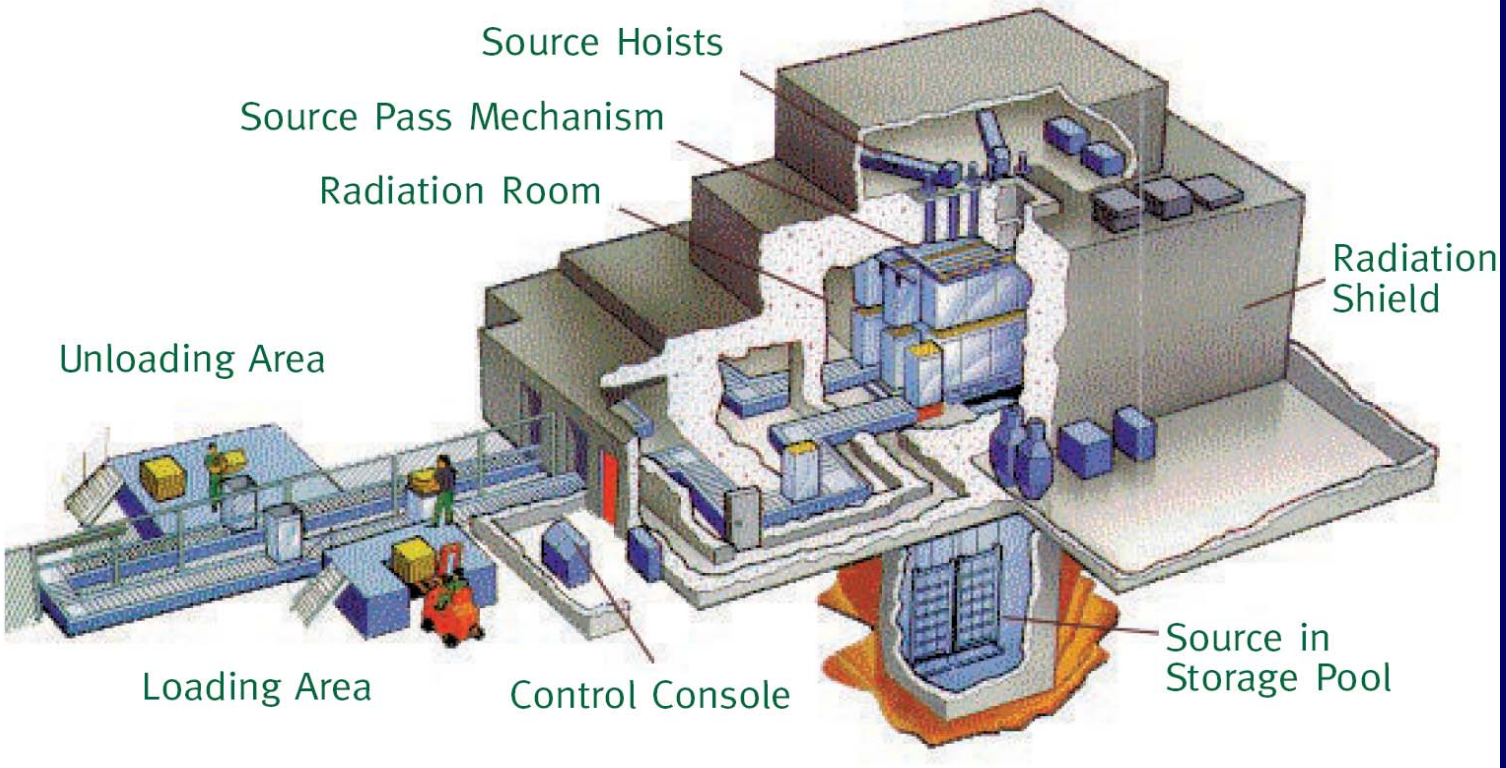
1 Gy (Gray) = 1 J of energy absorbed by 1 kg of matter

Food Irradiation Applications

Benefit	Dose (kGy)	Products
Low-dose (up to 1 kGy)		
(i) Inhibition of sprouting	0.05 - 0.15	Potatoes, onions, garlic, root ginger, yam etc.
(ii) Insect disinfestation and parasite disinfection	0.15 - 0.5	Cereals and pulses, fresh and dried fruits, dried fish and meat, fresh pork, etc.
(iii) Delay of physiological processes (e.g. ripening)	0.25 - 1.0	Fresh fruits and vegetables.
Medium-dose (1-10 kGy)		
(i) Extension of shelf-life	1.0 - 3.0	Fresh fish, strawberries, mushrooms etc.
(ii) Elimination of spoilage and pathogenic microorganisms	1.0 - 7.0	Fresh and frozen seafood, raw or frozen poultry and meat, etc.
(iii) Improving technological properties of food	2.0 - 7.0	Grapes (increasing juice yield), dehydrated vegetables (reduced cooking time), etc.
High-dose (10-50 kGy)		
(i) Industrial sterilization (in combination with mild heat)	30 - 50	Meat, poultry, seafood, prepared foods, sterilized hospital diets.
(ii)Decontamination of certain food additives and ingredients	10 - 50	Spices, enzyme preparations, natural gum, etc

Taken from: 'Facts about Food Irradiation'; The International Consultative Group on Food Irradiation (1999).

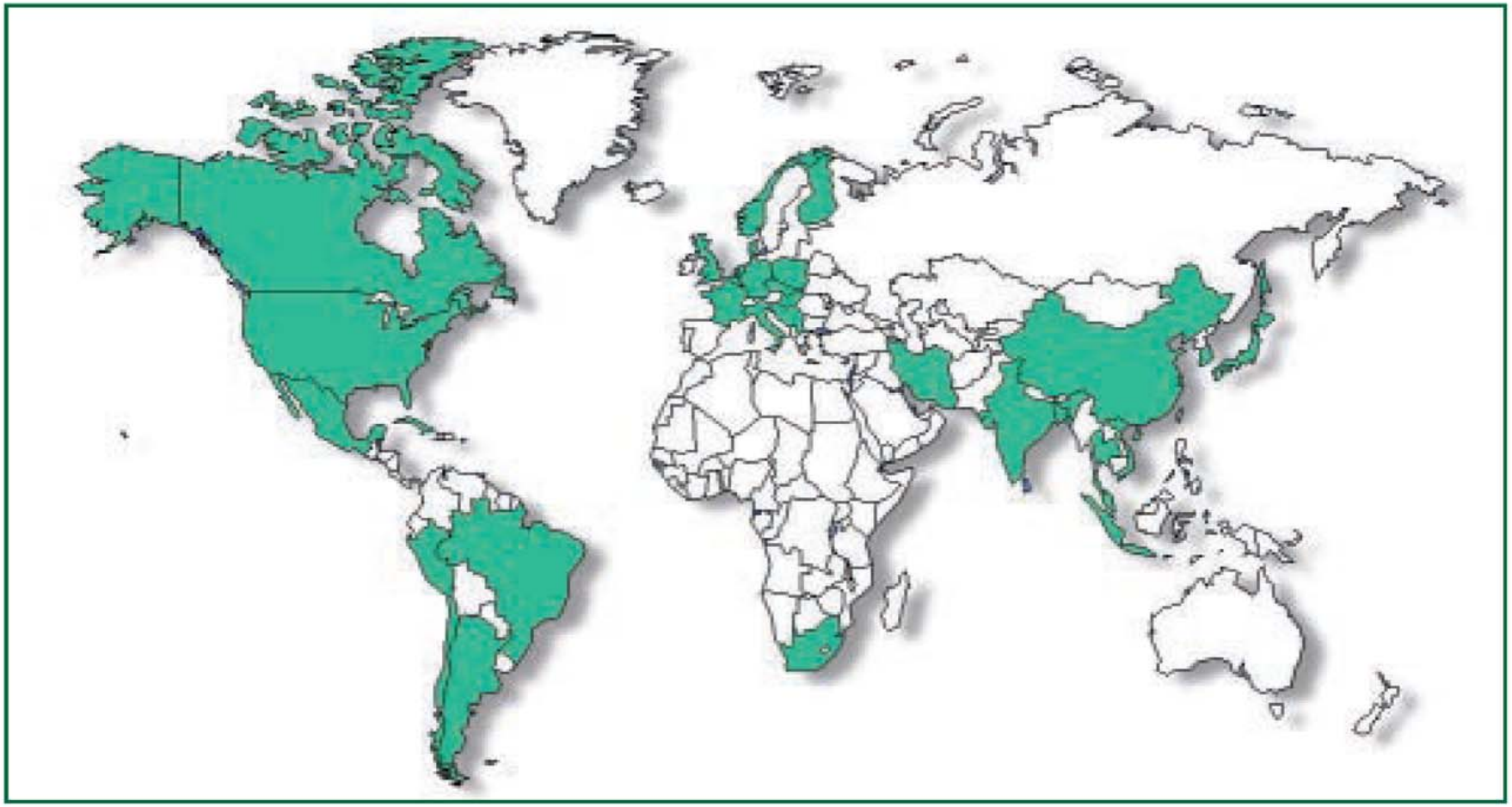
Typical layout of a food irradiation facility



Gamma Irradiator for food processing

Taken from: 'Facts about Food Irradiation'; The International Consultative Group on Food Irradiation (1999).

World-wide Utilization of Food Irradiation



Countries which apply food irradiation for commercial purposes

Do not yet apply food irradiation

Taken from: 'Facts about Food Irradiation'; The International Consultative Group on Food Irradiation (1999).

What kinds of irradiated foods are currently marketed?

- **Several irradiated foods are used by the food industry as ingredients:**
  - **e.g. irradiated spices, irradiated mechanically-deboned poultry meat**
- **Also retail products in various parts of the world:**
  - **Fruit (e.g. irradiated Hawaiian papaya – protection against fruit flies)**
  - **Spices, vegetable seasonings and associated products (South Africa, Belgium, China)**
  - **Frog legs (labelled 'treated by ionisation')**
  - **Garlic/ onions (to prevent sprouting; U.S. & China)**
  - **Chicken (U.S. – treatment against Salmonella)**
  - **Fermented Pork Sausages (Thailand; against Trichinella spiralis & Salmonella).**

Microbiological effects of irradiation

- **Microbes inactivated by damage to RNA, DNA, metabolic enzymes and cell membranes**
- **> 50 kGy required for complete sterilisation**
- **Such levels typically develop serious off-flavours**
  - **Dairy food particularly problematic**
- **Irradiation can usefully be applied as an anti-microbial agent where doses below 10 kGy are effective**
  - **E.g. 2.5 kGy will effectively eliminate *Salmonella***
- **Clostridium & other bacterial spores are resistant to low levels of irradiation (as with thermal processing)**
- **Risk: encourage growth of resistant pathogens by eliminating vulnerable spoilage organisms**
  - **Usual spoilage cues eliminated**

Radiation resistance of selected bacteria

Use "D_{10}" value – *Decimal Reducing Dose* (does required to reduce the population by 10): similar to the D_T value (*Decimal Reduction Time at fixed temperature T*) used in thermal processing

Table 2. Typical radiation resistances of some bacteria in non-frozen foods of animal origin (Farkas, 2001b)

Bacteria	D_{10} value (kGy)
Vegetative cells	
Aeromonas hydrophila	0.14–0.19
Bacillus cereus	0.17
Brucella abortus	0.34
Campylobacter jejuni	0.08–0.20
Clostridium perfringens	0.59–0.83
Escherichia coli (incl. O157:H7)	0.23–0.35
Lactobacillus spp.	0.3–0.9
Listeria monocytogenes	0.27–1.0
Moraxella phenylpyruvica	0.63–0.83
Pseudomonas putida	0.06–0.11
Salmonella spp.	0.3–0.8
Streptococcus faecalis	0.65–1.0
Staphylococcus aureus	0.26–0.6
Vibrio spp.	0.03–0.12
Yersinia enterocolitica	0.04–0.21
Bacterial spores	
Bacillus cereus	1.6
Clostridium botulinum types A and B	1.0–3.6
Clostridium botulinum type E	1.25–1.40
Clostridium sporogenes	1.5–2.2

From: Farkas, J. (2006). 'Irradiation for better foods'. Trends Food Sci. Technol. **17**: 148-152.

Impacts of irradiation on food quality

- **Irradiated foods are not themselves radioactive!!**
- **Irradiated foods contain elevated amounts of radiolytic products such as free radicals (reactive species with an un-paired electron)**
  - **However, food naturally contains background levels of radiation and radiolytic products**
- **Effects are dose dependent**
- **Can minimise sensory effects by**
  - **e.g. irradiating whilst frozen.**

What happens to food molecules?

Irradiation effects :

- **Direct** — **Ionisation & free radical formation due to bond breakage**
  - **The radicals are extremely short lived (< 10^{-5} s) but are sufficient to destroy bacterial cells**
- **Indirect** — **changes due to free radicals produced and further reactions**

Water (direct):

$H_2O \rightarrow e_{aq}^-$, $H^{\cdot}$, $OH^{\cdot}$, $H_2O_2^{\cdot}$

Lipids (vulnerable to free-radical damage):

- **non-oxidative**
- **oxidative**

What happens to food molecules?

Proteins:

- **Reduction in molecular weight => low Mol. wt peptides**
- **Enzyme denaturation (if >10 kGy)**

Carbohydrates:

- **Hydrolysis and oxidative degradation => reduction of molecular weight**
- **Lower saccharides may be oxidized => acids**

Vitamins:

- **Indirect, due to free radicals. Depends on water and oxygen content. Antioxidants such as vitamins C & E are vulnerable to radiolytic oxidation**
- **Cis-trans isomerisation (e.g. vitamin A)**

Summary: common radiolytic products of main food components

Food Component	*Typical Products*
1. Protein	**Low molecular weight peptide fragments. No persistence of free radicals. Low molecular weight sulphur compounds**
2. Carbohydrates	
starches	**glyceraldehyde, dihydroxyacetone, malic, formic acids**
sugars	**Low molecular weight oxygenated compounds**
3. Lipids	**Low molecular weight hydrocarbons, high molecular weight esters, CO_2, H_2, CO.**

Data from 'Radiation Chemistry of Major Food Components'. P.S. Elias & A.J. Cohen. Elsevier Biomedical Press. New York 1977.

Some biochemical effects of irradiation of fruits and vegetables

Irradiation response	Produce
Delayed ripening	Bananas, mangoes, papayas
Delayed ageing	Sweet cherries, apricots, tomatoes
Increased storage time	Strawberries, figs, pears
Irradiation damage	Avocadoes, nectarines, lemons, peaches
Accelerated ripening	Grapefruit, pineapples
No positive effect	Apples, plums, grapes

Data from 'Recent Advances in Food Irradiation'. P.S. Elias & A.J. Cohen. Elsevier Biomedical Press. Amsterdam 1977.

Typical vitamin losses (%) from food irradiation

- **Four vitamins are recognised as being highly sensitive to radiation:**
  - **B1, C (ascorbic acid), A (retinol) and E (α-tocopherol)**
  - **-However, B1 is more sensitive to heat**

Food	Vitamin Loss (%)					
	A	B1	B2	B6	C	E
Wheat		40		3		
Rice		20				
Beef	50	60	15	20		
Chicken	70	70	35	35		
Cod		47	2			
Mackerel		50		25		
Potatoes					30	
Tomatoes					14	
Nuts						25

Data from 'Recent Advances in Food Irradiation'. P.S. Elias & A.J. Cohen. Elsevier Biomedical Press. Amsterdam 1977.

Effect of irradiation on selected amino acids of Haddock fillets

	Amino acid content	
Amino acid	Not irradiated	Irradiated
Phenylalanine	3.93	3.63
Tryptophan	1.16	1.08
Methionine	2.99	2.85
Cystine	1.04	1.04
Valine	6.29	6.69
Leucine	8.03	8.25
Histidine	1.85	2.00
Arginine	5.34	5.56
Lysine	9.70	9.29
Threonine	4.87	4.58

Data from B.E. Proctor & B.S. Bhatia, *Food Technol. (Chicago)* 5, 357 (1950). Amino acid content expressed as parts of amino acid per 16 parts of nitrogen

Dose 53 kGy

Effect of Irradiation on viscosity and degree of polymerisation of potato amylose

Dose (kGy)	Intrinsic viscosity (ml g^{-1})	Degree of polymerisation
0	230	1700
0.5	220	1650
1	150	1100
2	110	800
5	95	700
10	80	600
20	50	350
50	40	300
100	35	250

From C.T.Greenwood & C. MacKenzie, *Die Stärke* **15**, 444 (1963).

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CARBOHYDRATE RESEARCH

Carbohydrate Research 282 (1996) 223–236

Effect of gamma irradiation on the macromolecular integrity of guar gum

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Received 7 August 1995; accepted 20 November 1995

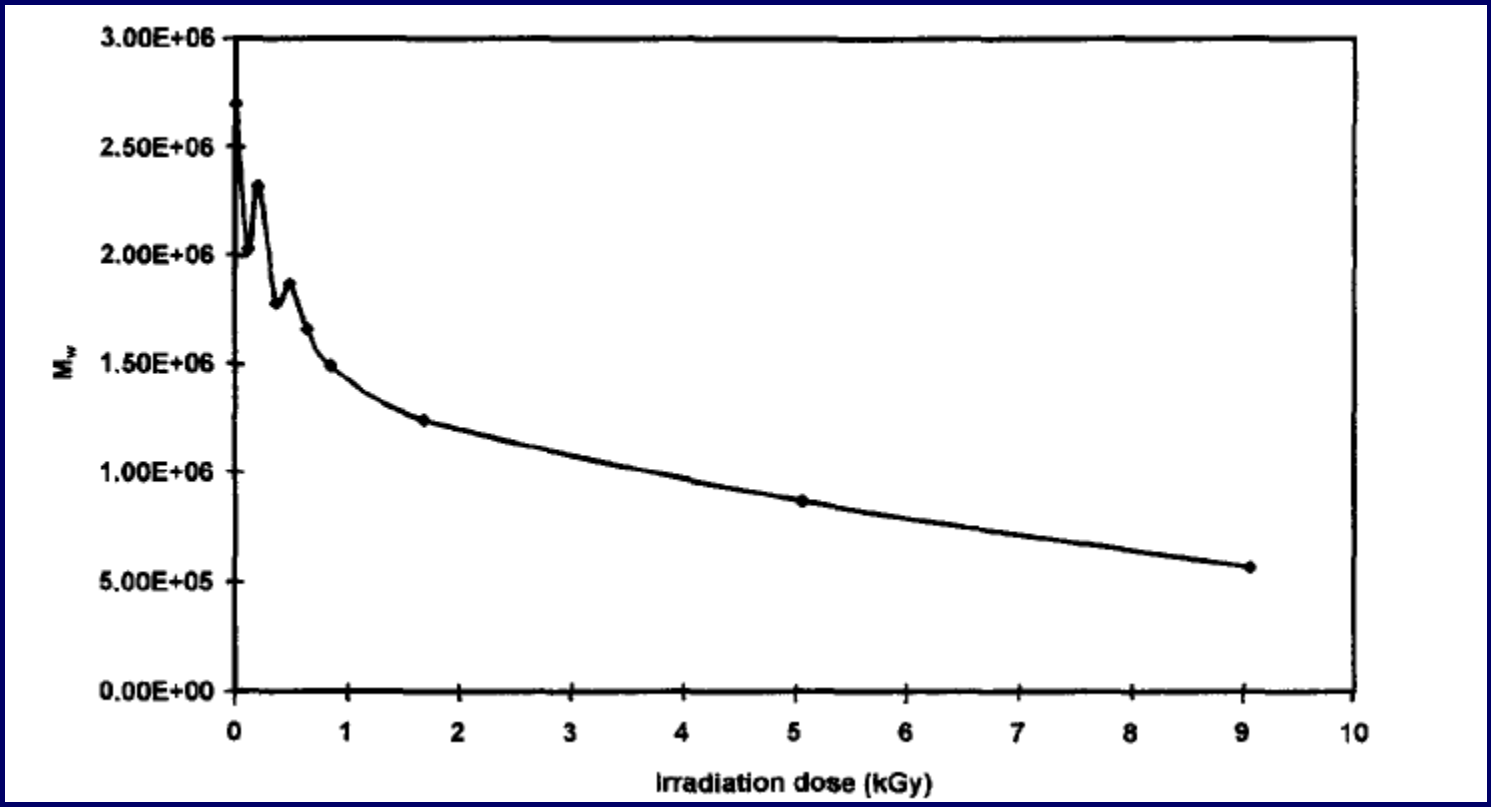
Light Scattering- “SEC MALLs”

Viscometry

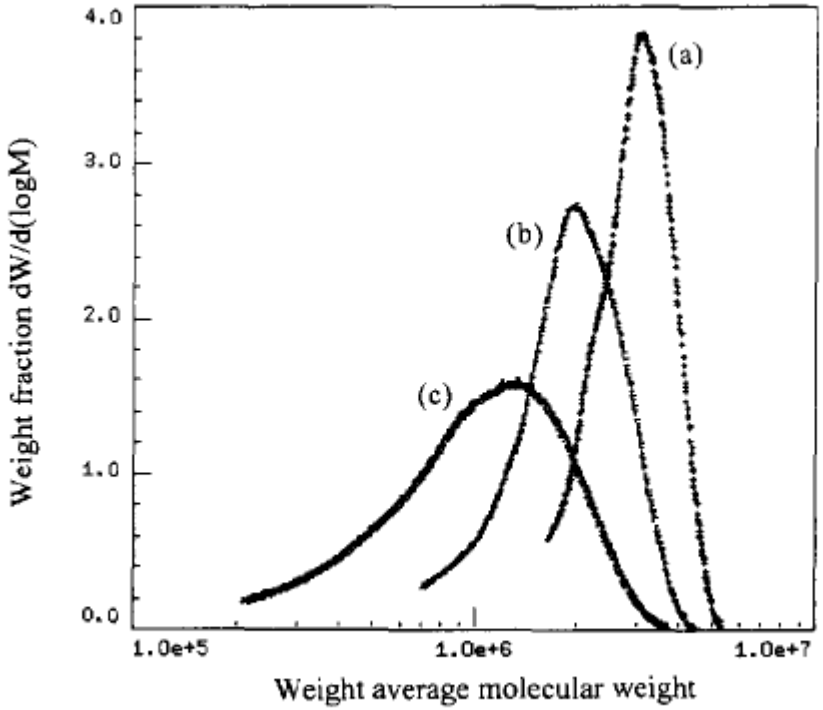
Analytical Ultracentrifugation

The SEC-MALLs data shows a clear drop in molecular weight (weight average) with increased dose

Light Scattering- “SEC MALLs”



... and a clear drop in the molecular weight distribution



Molar mass distributions of (a) non-irradiated, (b) 0.204 kGy, and (c) 1.700 kGy samples.

$G_{scission}$ values are a measure of the degree of degradation

$G_{(scission)}$ values for irradiated guar gum samples calculated using M_w from SEC/MALLS measurements

Radiation dose (kGy)	$G_{(scission)}$ value
0.113	10.34
0.204	2.96
0.373	5.00
0.498	3.14
0.649	3.37
0.860	3.40
1.700	2.48
5.072	1.49
9.071	1.48

$$G_{(\text{scission})} = \frac{S_{1000} \times 100}{\text{dose}\,(\text{eVg}^{-1}) \times \text{g}(1000\,\text{bonds})^{-1}}$$

$1\ \text{Gy} = 6.24 \times 10^{15}\ \text{eVg}^{-1}$.

The average number of scissions per gram of guaran is given by:

$$S = \left(\frac{dp_1}{dp_2}\right) \times \left(\frac{N}{dp_1 \times 512}\right)$$

where dp_1 = degree of polymerisation for non-irradiated guaran, dp_2 = degree of polymerisation of irradiated guaran, N = Avogadro's number, 512 g/mol = molar mass of guaran repeating unit.

The amount (in g) of guaran per 1000 glycosidic bonds is given by:

$$\text{g guaran}\,(1000)^{-1} = \frac{1000 \times dp_1 \times 512}{(dp_1 - 1)} \times N$$

The number of scissions per 1000 glycosidic bonds (S_{1000}) in guaran is given by:

$$S_{1000} \cong 1000\left[\frac{1}{dp_2} - \frac{1}{dp_1}\right]$$

Sedimentation velocity data shows unimodal “hypersharp” peaks – 7 scans shown taken at regular time intervals.

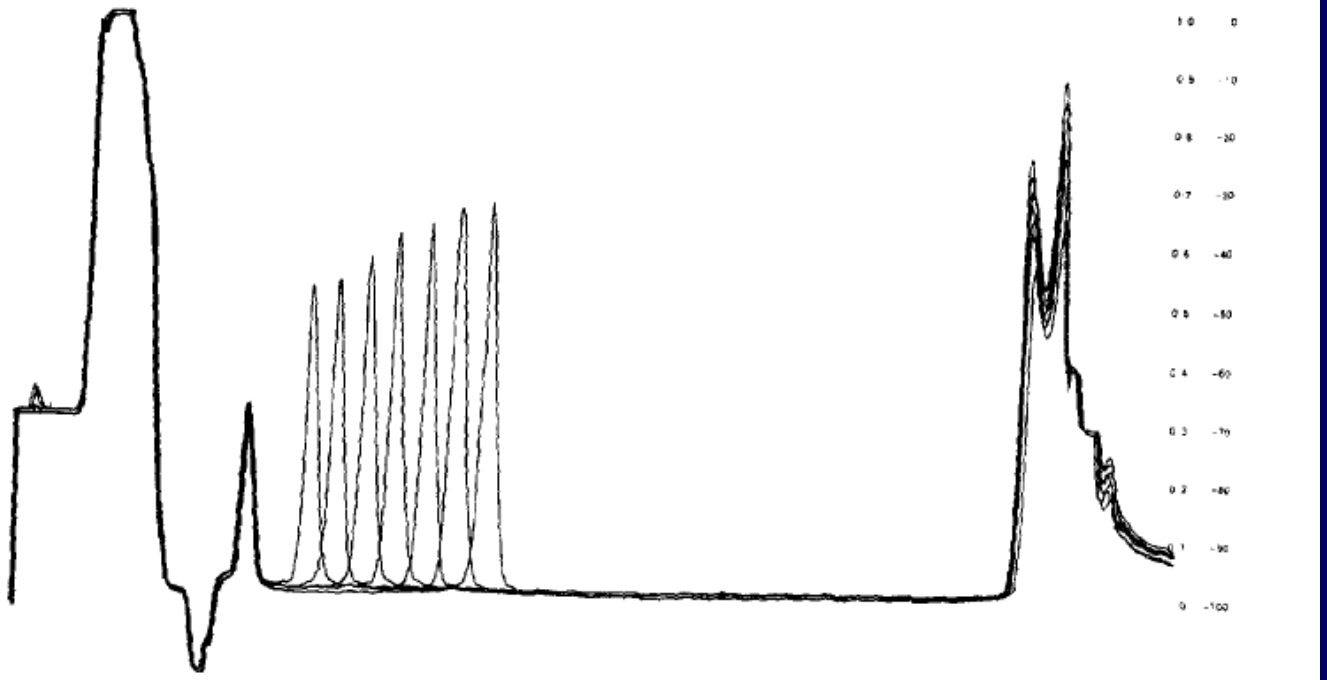


Fig. 8. Sedimentation velocity profiles from 0.649 kGy sample. Sample concentration = 1.8 mg/mL, rotor speed = 47,000 rpm, temperature = 20 °C.

Intrinsic viscosity (from capillary viscometry) also shows a strong decrease with dose

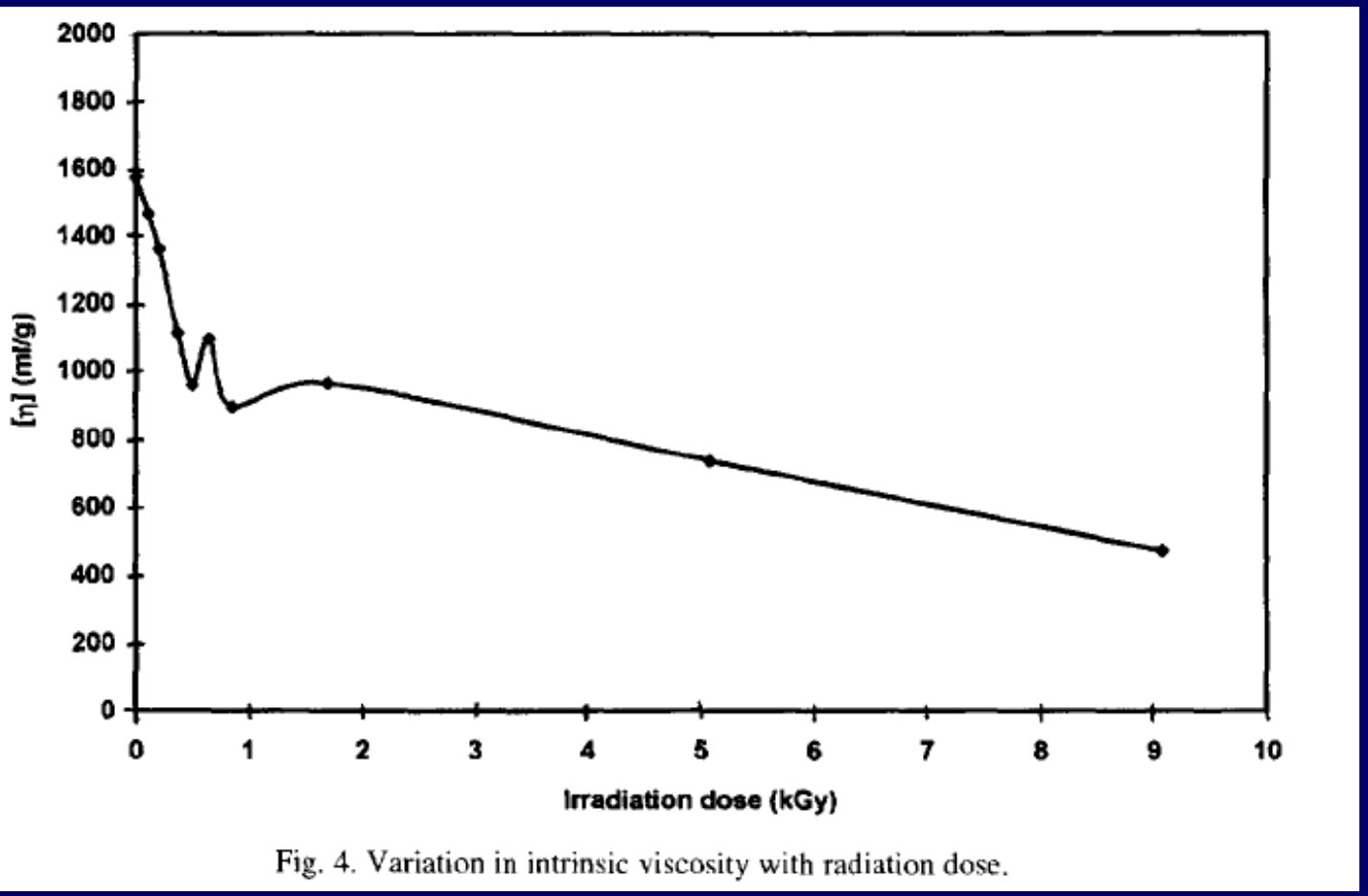


Fig. 4. Variation in intrinsic viscosity with radiation dose.

The data follows the same trend, including the zero shear viscmetry values (at 10mg/ml) from a Bohlin rheometer

Sample	$10^{-6} \times M_w$ light scattering	$[\eta]$ (mL/g)	η_0 (Pas)
Control	2.70	1576	21.4
0.113	2.03	1467	19.87
0.204	2.32	1360	12.91
0.373	1.78	1111	8.27
0.498	1.87	957	5.18
0.649	1.66	1092	4.64
0.860	1.49	894	5.17
1.700	1.24	964	1.71
5.072	0.866	736	0.46
9.071	0.565	471	0.12

[η], M data set allows evaluation of the Mark-Houwink conformation parameter a

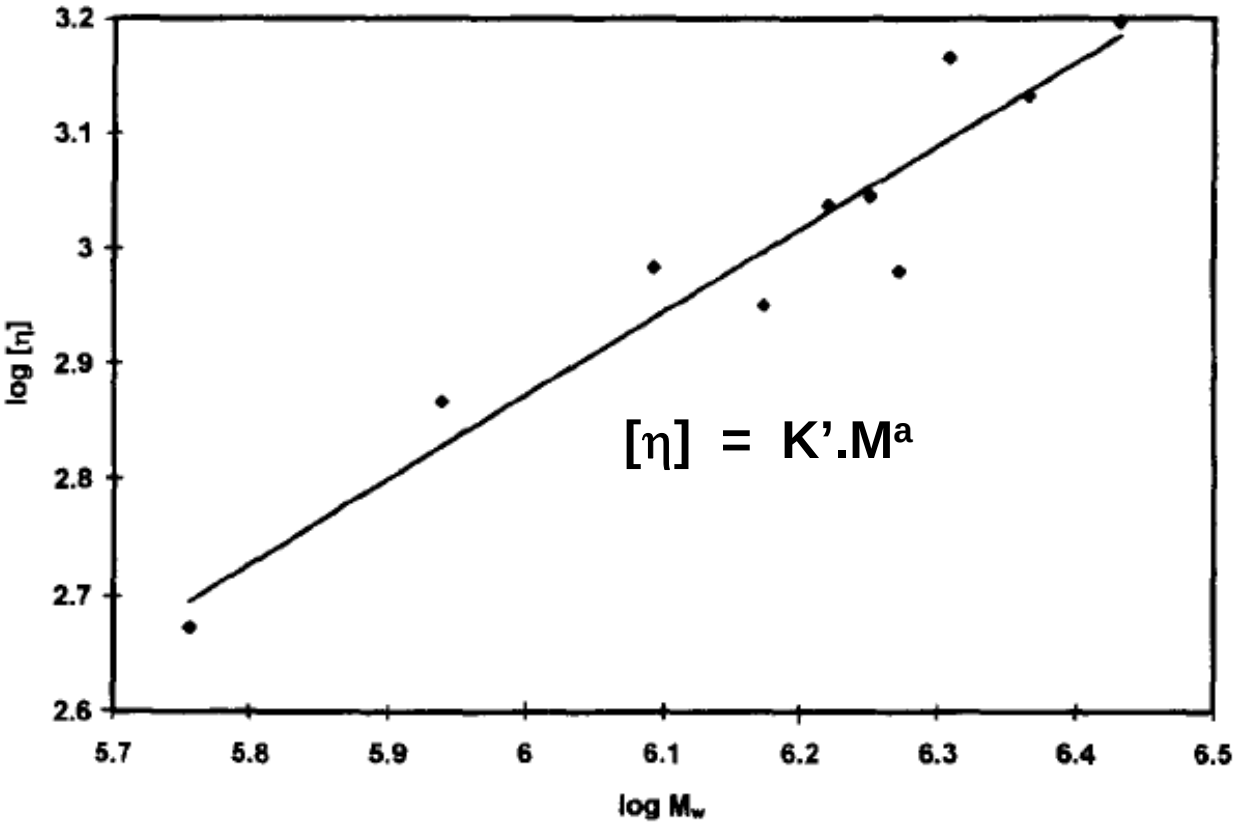


Fig. 5. Double-log plot of intrinsic viscosity versus molar mass.

a ~ 0.73 : flexible coil (same as native guar)

... and perhaps unsurprisingly, the solubility of guar increases with dose

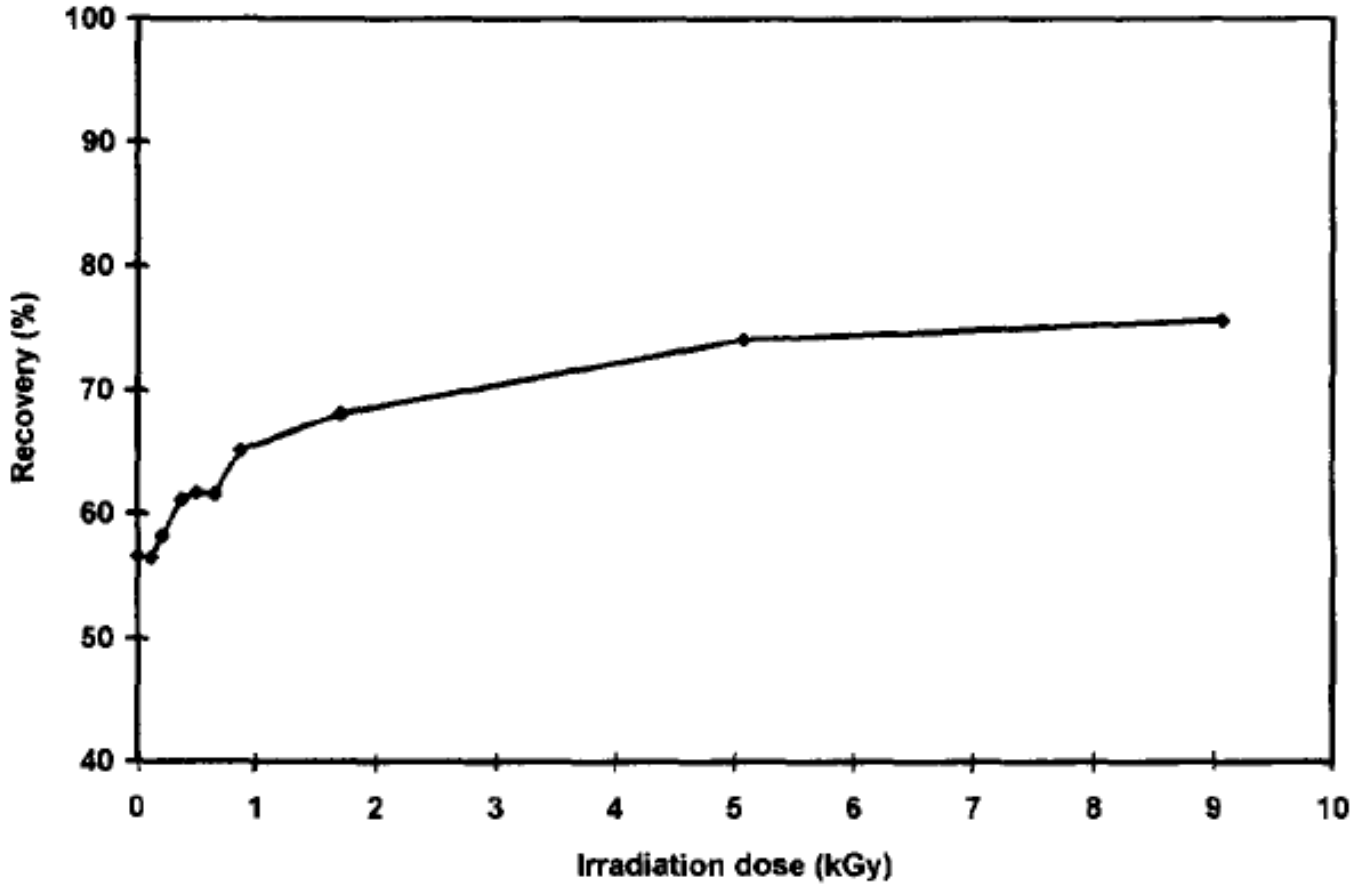


Fig. 10. Variation in guar gum solubility with irradiation dose.

… a more recent study shows similar effects for xyloglucan

Carbohydrate Polymers 74 (2008) 845–851

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journal homepage: www.elsevier.com/locate/carbpol

Global conformation analysis of irradiated xyloglucans

Trushar R. Patel [a,e], Gordon A. Morris [a,*], Anna Ebringerová [b], Melita Vodeničarová [c], Vladimír Velebný [c], Alvaro Ortega [d], Jose Garcia de la Torre [d], Stephen E. Harding [a]

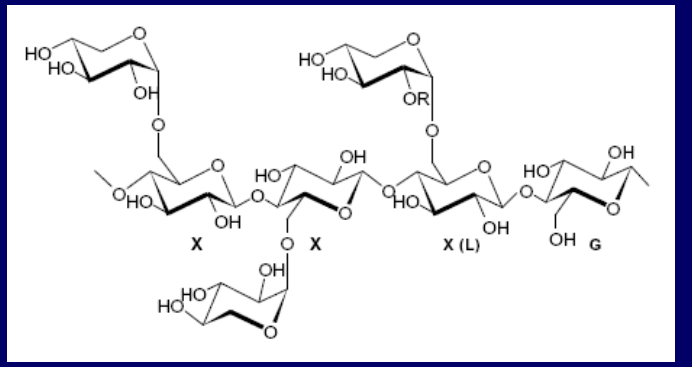

Table 1
Monosaccharide composition of native and γ-irradiated xyloglucans

Sample	Radiation (kGy)	Glc:Xyl:Gal (mole ratios)	Xyl:Gal
XG-0	0	1:0.68:0.32	2.1:1
XG-10	10	1:0.64:0.31	2.1:1
XG-20	20	1:0.63:0.31	2.0:1
XG-30	30	1:0.66:0.31	2.1:1
XG-40	40	1:0.64:0.32	2.0:1
XG-50	50	1:0.60:0.32	1.9:1
XG-70	70	1:0.78:0.36	2.2:1

... decrease in molecular weight of irradiated xyoglucans (SEC-MALLs technique)

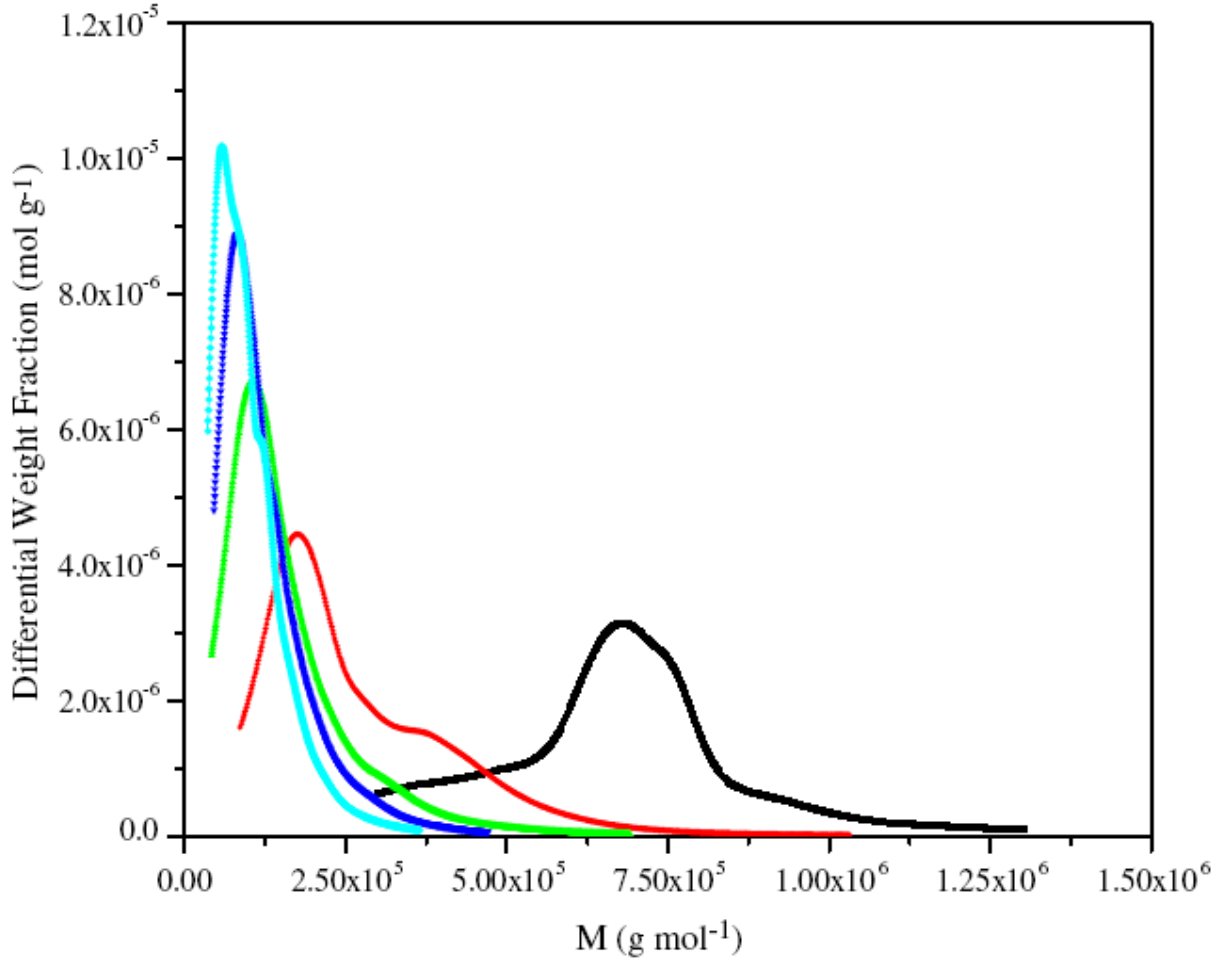

Fig. 3. Molecular weight distributions for xyloglucans: XG-0 (■), XG-10 (●), XG-20 (▲), XG-30 (▼) and XG-40 (◆).

... decrease in intrinsic viscosity and sedimentation coefficient as well

Hydrodynamic data for native and γ-irradiated xyloglucans

Sample	$s^{o}_{20,w}$ (S)	$[\eta]$ (mL/g)	$10^{-4} \times M_w$ (g/mol)	M_w/M_n
XG-0	7.21 ± 0.03	405 ± 35	70.0 ± 5.0	1.1 ± 0.1
XG-10	4.66 ± 0.03	210 ± 10	27.0 ± 1.0	1.3 ± 0.1
XG-20	3.10 ± 0.04	170 ± 10	15.8 ± 0.3	1.4 ± 0.1
XG-30	3.30 ± 0.01	140 ± 10	12.7 ± 1.0	1.3 ± 0.1
XG-40	2.82 ± 0.04	135 ± 5	9.7 ± 1.0	1.3 ± 0.1
XG-50	2.80 ± 0.08	100 ± 5	6.0 ± 0.4	1.3 ± 0.1
XG-70	2.61 ± 0.02	75 ± 5	4.5 ± 0.3	1.1 ± 0.1

... decrease in intrinsic viscosity and sedimentation coefficient as well

Hydrodynamic data for native and γ-irradiated xyloglucans

Sample	$s^{o}_{20,w}$ (S)	$[\eta]$ (mL/g)	$10^{-4} \times M_w$ (g/mol)	M_w/M_n
XG-0	7.21 ± 0.03	405 ± 35	70.0 ± 5.0	1.1 ± 0.1
XG-10	4.66 ± 0.03	210 ± 10	27.0 ± 1.0	1.3 ± 0.1
XG-20	3.10 ± 0.04	170 ± 10	15.8 ± 0.3	1.4 ± 0.1
XG-30	3.30 ± 0.01	140 ± 10	12.7 ± 1.0	1.3 ± 0.1
XG-40	2.82 ± 0.04	135 ± 5	9.7 ± 1.0	1.3 ± 0.1
XG-50	2.80 ± 0.08	100 ± 5	6.0 ± 0.4	1.3 ± 0.1
XG-70	2.61 ± 0.02	75 ± 5	4.5 ± 0.3	1.1 ± 0.1

... and again we can combine these data to see if the conformation and chain flexibility has been altered

... comparison of the flexibility parameter L_p

Individual estimates of L_p/M_L for each irradiated xyloglucan. Corresponding persistence lengths also given for $M_L \sim 537\ g\ mol^{-1}\ nm^{-1}$

Sample	L_p/M_L ($nm^2\ mol\ g^{-1}$)	L_p (nm)
XG-0	0.011 ± 0.002	6 ± 1
XG-10	0.011 ± 0.002	6 ± 1
XG-20	0.017 ± 0.002	9 ± 1
XG-30	0.011 ± 0.002	6 ± 1
XG-40	0.015 ± 0.002	8 ± 1
XG-50	0.011 ± 0.002	6 ± 1
XG-70	0.011 ± 0.004	6 ± 2
Overall	0.013 ± 0.002	7 ± 1

... conclusion is that gamma irradiation causes chain scisission but no measurable change in chain flexibility

Advantages/ applications of food irradiation

- **No competitive alternative for some food products such as spices and tropical fruits**
- **Prolonging shelf-life**
- **Alternative to chemical preservatives**
- **No heating => freshness and physical states maintained (fruits, vegetables and frozen commodities)**
- **Nutrient (vitamins) loss comparable to loss during cooking (dependent on dose)**
- **Reduces food waste**
- **Packaged and frozen foods may be treated**

Disadvantages / concerns about food irradiation

- **The “not known” syndrome**
- **Kills bacteria but does not remove already existing toxins => Warning (colour + odour) signs could be eliminated**
- **Loss of flavour + generation of odor**
- **Some molecular and macromolecular degradation – e.g. guar study**
- **Cost of irradiation plants (particularly in the developing world e.g. $ 5 million for a cobalt-60 food irradiation plant)**
- **Psychological concern => Market affected. Some paralels with the GM-food debate**
- **Hard to evaluate risk of forming mutant strains of bacteria**

Food and Biopharma Processes imposing stresses on macromolecules:

- Thermal Processing
- Irradiation
- Freeze-thaw
- Spray drying,
- Filtration,
- Extrusion,
- Lyophilisation

Food and Biopharma Processes imposing stresses on macromolecules:

- Thermal Processing ✓
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Freeze thaw processing – effect on an antibody

The Effect of a Point Mutation on the Stability of IgG4 as Monitored by Analytical Ultracentrifugation

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ABSTRACT: There is presently considerable interest in the state of aggregation and biophysical integrity of antibody preparations, and recent advances in the analysis of data from the analytical ultracentrifuge renders it a powerful probe of these stability phenomena, under both storage and freeze-thaw conditions. Solutions of a wild-type IgG4 antibody and a single amino acid hinge mutant IgG4m (serine residue 241 converted to proline) were exposed to different accelerated stress conditions, namely (i) elevated temperature storage for various periods (up to 59 days at 37°C) or (ii) a series of freeze-thaw cycles (storage at −80°C then incubation at 20°C for 1 h under different conditions). Analysis using the nondisruptive probe of sedimentation velocity in the analytical ultracentrifuge indicated that for both antibodies the monomer was always the most common species present whatever storage regime had been used. Sedimentation coefficient distribution analysis showed that other higher oligomer species and half-antibodies were present, and appeared to be not in chemical equilibrium with each other. Solution heterogeneity was found to increase considerably with treatment for both native and hinge-mutant antibodies although the latter appeared to be more resistant to freeze-thaw-induced aggregation. J Pharm Sci 97:960–969, 2008

Keywords: sedimentation coefficient distribution; serine-proline mutation; freeze-thaw; aggregation; half-antibody

Freeze thaw processing – effect on an antibody

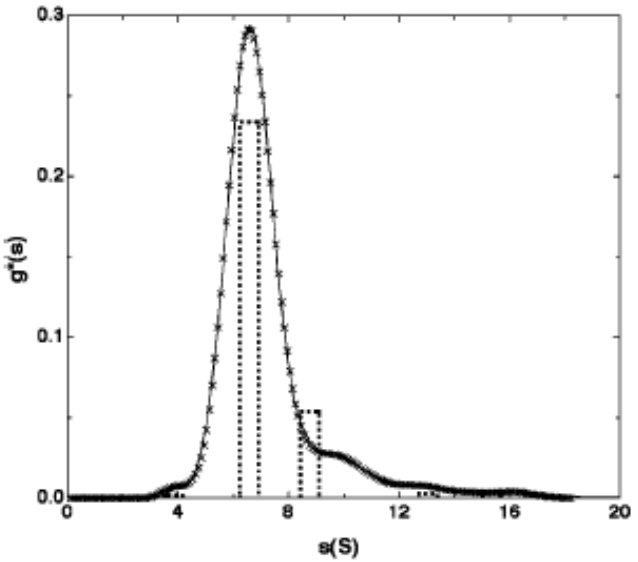


Figure 1. An example of the apparent sedimentation coefficient distribution analysis of IgG4wt. Multi-Gaussian fitting of the least-squares ls-$g^*(s)$ distribution (× experimentally obtained distribution—multi-Gaussian fit) for a 1.6 mg/mL IgG4wt solution obtained from the stock solution after five cycles of freeze-thaw treatment. Five species were resolved by the analysis, the proportions of the species represented in the bar chart, are 0.5% (of the total amount of sedimenting material determined by UV absorbance) sedimenting at 3.83S, 80.0% sedimenting at 6.57S, 19.4% sedimenting at 8.76S, 0.8% sedimenting at 13.1S and 1.3% sedimenting at 15.4S.

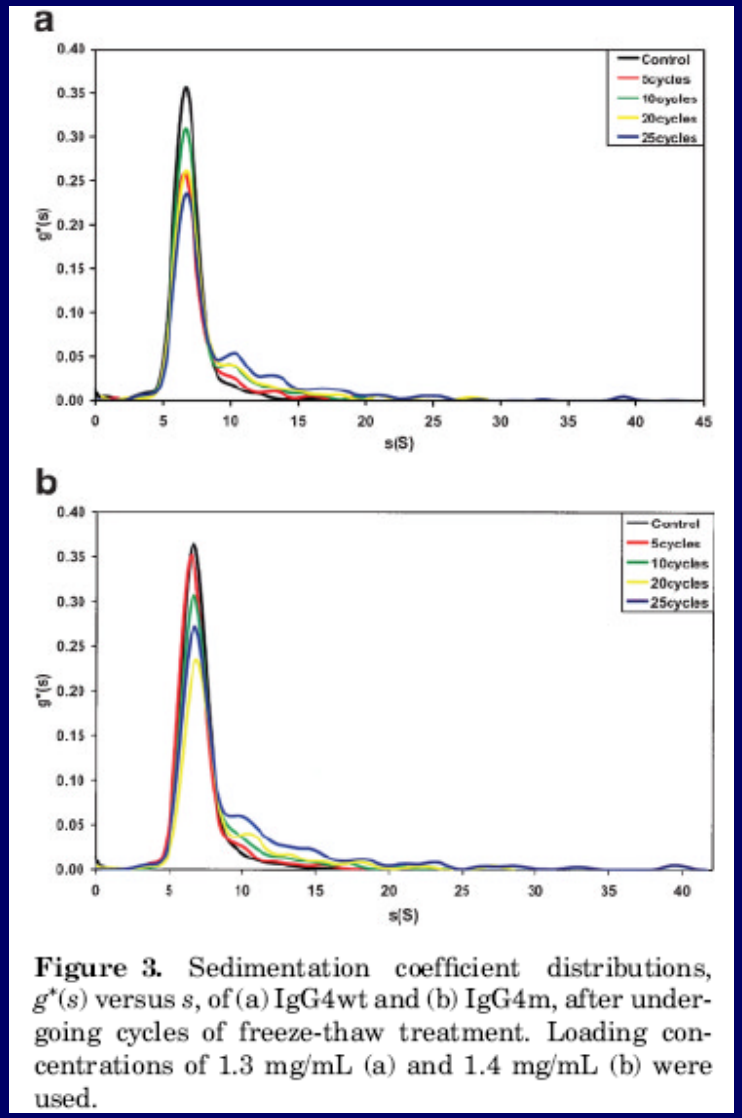
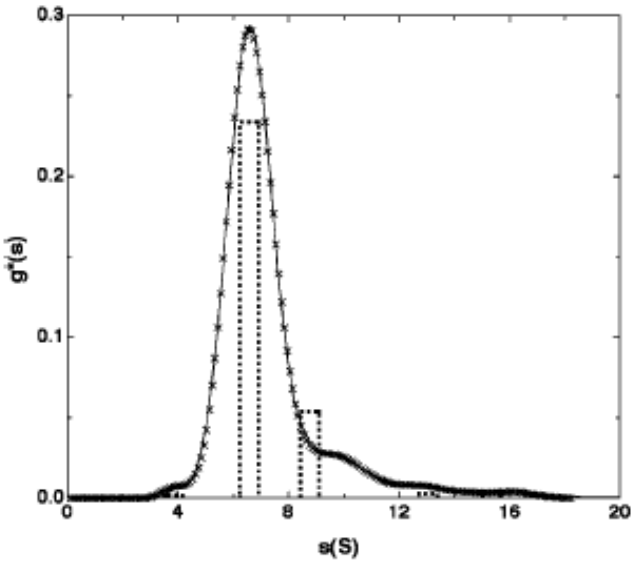


Figure 3. Sedimentation coefficient distributions, $g^*(s)$ versus s, of (a) IgG4wt and (b) IgG4m, after undergoing cycles of freeze-thaw treatment. Loading concentrations of 1.3 mg/mL (a) and 1.4 mg/mL (b) were used.

- Increase in proportion of aggregate relative to monomer

Freeze thaw processing – effect on an antibody



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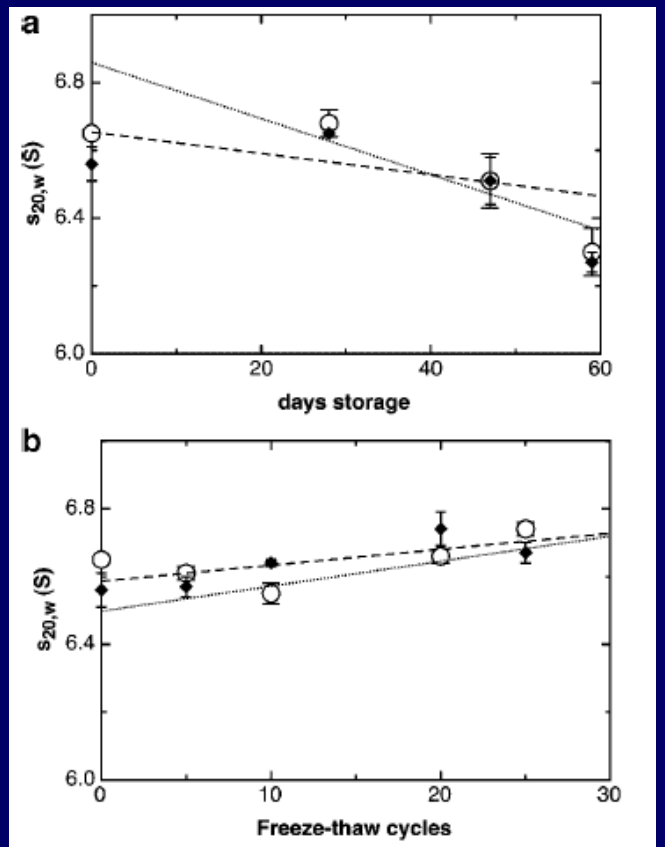

Figure 6. (a) Changes in the sedimentation coefficient of the IgG4wt monomer (open circle, dashed line) and of the IgG4m monomer (closed diamond, faint line) after 37°C storage. The standard error of the estimate of the sedimentation coefficient obtained at each condition is shown. (b) Changes in the sedimentation coefficient of the IgG4wt monomer and of the IgG4m monomer after cycles of freeze-thaw treatment. Other details as in (a).

- Change in sedimentation coefficient and its dependence on concentration: conformation linked effect on aggregation?

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