

Application of ionizing radiations to produce new polysaccharides and proteins with enhanced functionality

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Abstract

Treatment of polysaccharides with ionizing radiation either in the solid state or in aqueous solution leads to degradation. Using a mediating alkyne gas during the radiation treatment prevents the degradation of natural and synthetic polysaccharides and enables the introduction of different molecular and functional characteristics, as previously achieved with synthetic polymers. The molecular weight can be increased and hydrogel forms of the polysaccharides or proteins can be produced, probably by a cross-linking mechanism involving addition of C–C bond between two chains. The product has higher viscosity and is more elastic than the original material and, therefore, gives enhanced functionality. Gum Arabic (*Acacia senegal*) irradiated for 6.2 kGy is used here to demonstrate the enhanced emulsification performance and stability compared with the original material.

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1. Introduction

Polysaccharides are natural polymers derived from vegetable, animal, or microbial origin. They are naturally present or added to control the functional properties of aqueous solution. The functional properties of polysaccharides and proteins are directly linked to their structure which determines how they interact with water. There can be no clearer demonstration of this than the comparison of amylose and cellulose. The former is made up of α -1-4 D-glucose chains and the latter of β -1-4 D-glucose chains. Although made up of the same constituent monosaccharide the different stereochemical linkage leads to an entirely different conformation and hence physical proper-

ties. Amylose, in physical terms, is a water-soluble weak powder and cotton cellulose is a water-insoluble strong fibre. The natural built in variability in polysaccharides presents a challenge for supplying material of consistent quality and functionality. For example, the composition and molecular weight of acacia gum might depend on factors such as location and age of the trees [1]. Thus numerous attempts have been made to control and improve the functionality of such polysaccharides by chemical cross-linking. For example, hyaluronan (HA), a linear polysaccharide of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine linked β (1–4) has been cross-linked with formaldehyde or divinylsulfone [2], disulfide bonds [3], diepoxybutane [4], polyglycerol polyglycidylether [5] and adipic acid dihydrazide (ADH) [6]. These cross-linking procedures are either aimed at increasing the molecular weight or producing hydrogel forms. The hyaluronan products have been used for the treatment of

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osteoarthritis [7], ophthalmic surgery [8], tissue engineering [9] and drug delivery [6].

The application of radiation to process synthetic polymers to introduce structural changes by cross-linking and special performance characteristics is now a thriving industry [10]. In contrast treatment of polysaccharides and other natural polymers with ionizing radiation either in the solid state or in aqueous solution leads to degradation [11,12]. Recently, it has been shown that irradiation of highly concentrated solutions (paste-like state) of carboxymethyl cellulose (CMC) [13] or gum arabic [14] can lead to cross-linking without the need for any additives. Therefore, a method to modify structure, without introducing new chemical groupings, could prove of advantage, particularly if the process could be achieved in the solid state. This has been possible in synthetic polymers by exposure to high energy ionizing radiation, arising mainly through the pioneering work of Charlesby [10]. The method is now routinely used for the cross-linking of polymers [15]. Polymer chains can be joined and a network formed. The method is used for crystal lattice modification for semiconductors and gemstones, etc., by which the crystalline structure of a material is modified. The sheathing on wire and cable is routinely cross-linked with radiation to improve a number of important properties and radiation cross-linked polymers are commonly used to make heat-shrinkable tubing, connectors, and films.

This paper illustrates the use of a method which allows the controlled modification of the structure of polysaccharide and other related materials in the solid state using ionizing radiation in the presence of a mediating alkyne gas [16]. The range of materials modified by this process includes non-substituted polysaccharides extending over both plant and animal derived polysaccharides, whether charged or uncharged. The process has also been applied to proteins either directly derived from animal connective tissue sources such as collagen, gelatin, and from human and animal products, such as casein, combinations of one or more such polysaccharides with proteins of plant origin, biological tissues and materials derived therefrom used for tissue replacement and transplantation.

2. Experimental

2.1. The process

The dry solid material to be processed is first contained in a plastic bag, as is used in the packaging equipment for maintaining an inert atmosphere above a food product to prevent deterioration. Provision is available for evacuating the air and then replacing the air with the selected mediating gas, which in the process is an alkyne, usually acetylene. The bag is sealed with the gas *in situ* under normal pressure and submitted to ionizing radiation, usually gamma radiation. A typical source used for the experimental work was a gamma radiation source of 50 kCi, delivering a dose rate of 10 kGy/h at a temperature of 30 °C. Later, in larger

production batches commercial gamma processing plants were employed when upwards of kilograms could be processed. The effect of dose, but not dose rate is critical to control the degree of modification required. Any residual traces of alkyne gas remaining can be removed by again evacuating and replacing the atmosphere with nitrogen. The minimum absorbed radiation dose may vary from 1 to 50 kGy, depending on the structure of the biopolymer, whether branched or long-chain nature of the product desired, whether of increased molecular weight to form a readily water soluble product or to form either a hydrogel or a membrane product. As a general guide, highly branched polysaccharide structures can produce a 4-fold increase in molecular weight with doses up to 6 kGy and hydrogels with doses up to 50 kGy, whereas straight chain structures can yield a similar change with doses as low as 1–3 kGy. Proteins require doses up to 25 kGy to achieve a similar result. Blends and combined adhesive systems require careful dose selection according to the composition of the systems.

2.2. GPC-MALLS

Gel permeation chromatography coupled on line to a multi-angle laser light scattering detector, refractive index and UV was used in this study to determine the molecular weight and distribution. The GPC-MALLS system has already been described [17]. Briefly, the system comprised of solvent delivery system connected to a manual Rheodyne Model 7125 syringe loading sample injector equipped with a 100 μ l sample loop, DAWN DSP laser light scattering photometer equipped with a 632.8 nm He–Ne laser (Wyatt Technology Corporation, USA), a concentration dependent detector Wyatt Optilab DSP interferometric refractometer operated at 632.8 nm equipped with a 10-mm P100 cell (Wyatt Technology Corporation, USA) and a UV detector (Agilent Technologies 1100 series). Data accumulation for the detectors used Wyatt Technology ASTRA 4.5 software. All measurements were carried out at room temperature.

In the following text and Tables the expressions M_w and M_n are used for the weight and number average molecular weights and M_w/M_n for the polydispersity index (M_w/M_n) and R_g for the root mean square radius of gyration, % mass which indicate the mass recovery of the material after passing through column using a given refractive index increment (dn/dc) for a polymer solvent system.

2.3. Rheological measurements

Rheological measurements were carried out using a Carimed AR 550 stress controlled rheometer (AR 550) fitted with cone and plate geometry with a cone diameter of 4 cm and an angle of 2°. Dynamic rheological measurements, to determine the elastic modulus (G'), viscous modulus (G'') and dynamic viscosity, were performed in the frequency range of 0.1–10 Hz. The temperature of the

sample was controlled within 0.1 °C using a Peltier element. The actual concentration was calculated based on loss on drying. The solutions were prepared in water containing 0.005 w/v% NaN_3 as a preservative unless otherwise stated. The samples were tumble mix for 24 h and measurements were carried out at 25 °C unless otherwise stated.

2.4. Emulsification measurements

Oil in water (O/W) emulsion was made at 20% gum:20% oil phase. MCT was selected as dispersed (oil) phase, since it dissolves many lipophilic substances, the density (0.95 g/ml) is close to that of water, it has no smell, and is stable to oxidization, therefore, is suitable for preparing basic emulsions. The test material was dissolved in distilled water and after complete dissolution citric acid (to adjust the pH to 4), sodium benzoate (as preservative), and MCT were added. The composition of the mixed solution was: 20 w/w% gum arabic, citric acid 0.12 w/w%, sodium benzoate 0.13 w/w% and MCT 20 w/w%. The mixed solution was vortexed vigorously for 1 min. Emulsions were prepared using a Polytron PT-2100 homogenizer at 26,000 rpm for 3 min in an ice water bath. PTDA2112/2EC (9 mm tip diameter, tip speed 10.4 m/s) was used as dispersing tool. The emulsion was then homogenized using a Nanomizer (NM2-L100-D07, Collision type S generator, Yoshida Kikai Co. Ltd., Japan) three times at 75 MPa.

Particle size distribution of the emulsions was analyzed using a Mastersizer 2000 laser diffractometer (Malvern Instruments, UK). Distilled water was used as dispersant, and 1.450 ml/g was used as the refractive index for MCT. The volume weighted mean (D4,3) is the mean diameter over the volume distribution and % of particles greater than 1 μm were used to compare the emulsions performance.

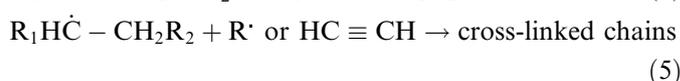
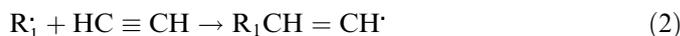
Prepared emulsions were subjected to acceleration test by storing at 60 °C (Gallenkamp, OVA031.XX1.5) for 3 and 7 days. Subsequently the particle size was then re-measured by a Mastersizer 2000. The difference between the initial droplet size and after acceleration test was used to judge the emulsion stability.

3. Results and discussion

3.1. Polysaccharides

Previously we have shown that γ -irradiation of polysaccharides such as pullulan, dextran, protein and interactive blends (two polysaccharides) thereof can be increased in molecular weight, probably by a cross-linking mechanism [18]. Eqs. (1)–(5), illustrate the proposed cross-linking mechanism between two chains. For ease of presentation we labelled the two chains as R_1H and R_2H . The direct radiation action forms a free radical (designated here as $R_1\cdot$) which then adds to the acetylene to give a radical with a double bond. This addition to the acetylene is slow and

the reactive radical with a double bond abstracts hydrogen atom from a nearby polysaccharide chain to give two radicals, one on the original acetylene adduct and one on a nearby polysaccharide chain ($R_2\cdot$). These recombine to give a cross-linked stable radical. This radical has fair degree of mobility and either recombines with acetylene, radical generated as a result of the action of ionizing radiation or another similar radical. This process occurs also with polyethylene in radiolysis and for many polymers during photolysis [19]



Here we describe the effect of radiation modification of arabinogalactan protein complex (*Acacia senegal* referred also to as gum arabic), in the solid state. Gum arabic is a complex polysaccharide consisting of D-galactopyranose (~44%), L-arabino-pyranose and furanose (~25%), L-rhamnopyranose (14%), D-glucuropyranosyl uronic acid (15.5%), 4-O-methyl-D-glucuropyranosyl uronic acid (1.5%) and contains a small amount (~2%) of protein as an integral part of the structure [20]. The process allows us to increase the molecular weight, to continue the process to produce a hydrogel and control the viscoelasticity of the product.

Gel permeation chromatography coupled on line to a multi angle laser light scattering detector (GPC-MALLS) is currently the best available technique for the absolute determination polysaccharide molecular weights and their distribution. A typical elution profile of gum arabic after being separated by GPC is shown in Fig. 1. The elution profile of *A. senegal* and determination of the weight average molecular weight (M_w) for the whole gum and for two components have been interpreted [17,21]. The first peak is assigned to the high molecular weight arabinogalactan

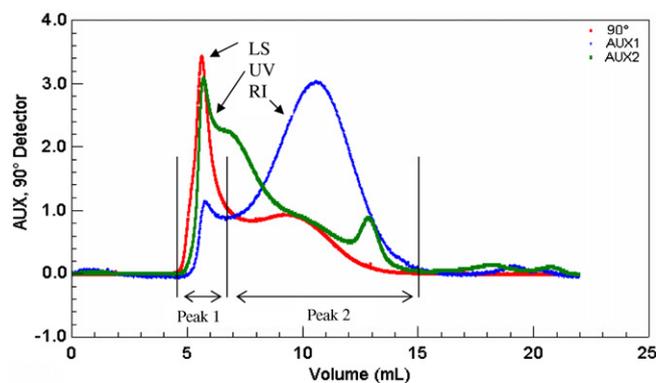


Fig. 1. GPC elution profile monitored by light scattering, refractive index and UV for control gum arabic.

Table 1
Molecular weight (g/mol) of control and irradiated gum arabic, in the presence of acetylene, determined by GPC-MALLS

Dose (kGy)	Processed as one peak				Processed as two peaks			
	M_w (g mol)	mass %	M_w/M_n	R_g (nm)	M_w (g/mol)	mass %	M_w/M_n	R_g
Control	$5.99 \pm 0.2 \times 10^5$	107	2.24	25	$2.34 \pm 0.09 \times 10^6$	14.5	1.49	35
6.2	$1.48 \pm 0.08 \times 10^6$	106	3.73	56	$3.19 \pm 0.08 \times 10^5$	93.1	1.35	–
					$5.26 \pm 0.30 \times 10^6$	22.7	1.59	63
10.5	$1.98 \pm 0.2 \times 10^6$	91.5	4.26	81	$4.61 \pm 0.24 \times 10^5$	84.1	1.43	24
					$6.88 \pm 0.7 \times 10^6$	20.8	1.78	90
26.5	$2.06 \pm 0.14 \times 10^6$	74	4.67	75	$5.32 \pm 0.36 \times 10^5$	70.5	1.44	33
					$7.75 \pm 0.56 \times 10^6$	15.4	1.94	83
					$5.21 \pm 0.28 \times 10^5$	57.5	1.46	25

M_w means weight average molecular and is given in column 2. % mass means the recovered mass which is calculated using a dn/dc value of 0.141 g/ml, R_g is the RMS-radius of gyration. M_w processed as two peaks means that the AGP peak was processed as one peak and the rest of the gum (AG + GP peaks) is processed as the second peak.

protein (AGP) and integration of the area under the peak gives its mass % with respect to the total injected mass. The second peak is broader with lower response is associated with the arabinogalactan (AG) component and it accounts for the rest of the gum (~90%). The molecular weight data are tabulated in Table 1. The weight average molecular weight for the starting material was 5.9×10^5 (g/mol). The AGP content was 14.5% of the total gum by mass with molecular weight of 2.3×10^6 (g/mol). The results are typical *A. senegal var. senegal* in the spray dried form. Upon irradiation in the presence acetylene to a dose of 6.2 kGy the weight average molecular weight is increased 2.5 times compared to the control ($M_w = 1.5 \times 10^6$ g/mol). This increase is also reflected in the % of AGP which is increased to 22.7% and the average molecular weight is more than doubled to 5.2×10^6 (g/mol). The radiation dose of 6.2 kGy achieved this increase in molecular dimensions with the sample retaining complete solubility. As irradiation is continued to higher doses, the product becomes too large to maintain complete water solubility and a hydrogel is formed. Mass recovery provides an indication of the amount of hydrogel formed since it is retained on the filter (0.45 μ m) used to clarify the solution prior to injection on to the column. The molecular weight of the whole gum and the AGP is increased more than three times to the control. The amount of these gel particles is 9% and 26% at doses of 10 and 26 kGy,

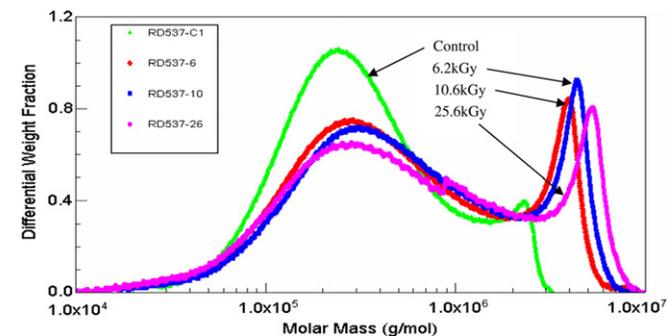


Fig. 2. Molecular weight distribution of control and irradiated spray dried *Acacia senegal*. Irradiation was carried out in the presence of acetylene.

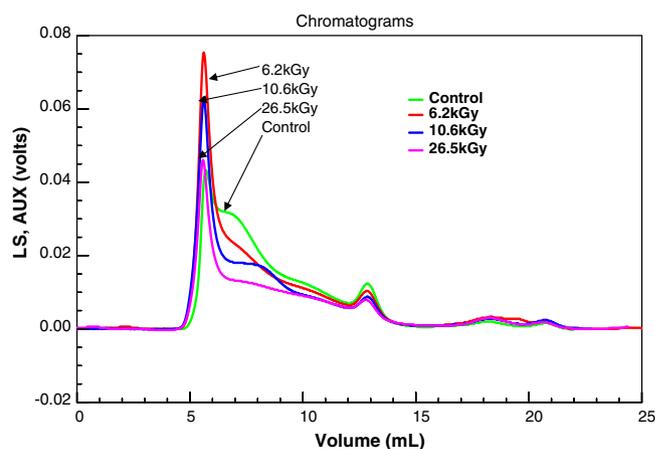


Fig. 3. UV profile of control and irradiated samples of spray dried *Acacia senegal*. Irradiation was carried out in the presence of acetylene.

respectively (Table 1). The changes in molecular weight parameters are illustrated in the molecular weight distribution (Fig. 2) which shows the build up of the AGP fraction and the reduction in AG fraction as a result of cross-linking.

Fig. 3 further shows the changes outlined above of the overall protein distribution between the main three components (AGP, AG, and GP) for the control and treated samples. Previously it was shown that the emulsification performance of *Acacia* gums is dependent on the high molecular weight arabinogalactan protein component (AGP) [21]. The radiation process allows us to increase the proportion of this component and so enhance the emulsification functionality. The enhanced functionality is demonstrated here by comparing the control with the irradiated sample for 6.2 kGy since it gave completely water soluble sample. The volume weighted mean (D4,3) and % particles greater than 1 μ m for initial and accelerated emulsions for 3 and 7 days at 60 $^{\circ}$ C are shown in Figs. 4 and 5, respectively. The results given in the latter Figures show the initial emulsification performance is almost similar but greater emulsion stability was achieved for the irradiated samples compared to the control. The stability of the emulsion is directly related to greater oil surface coverage by the

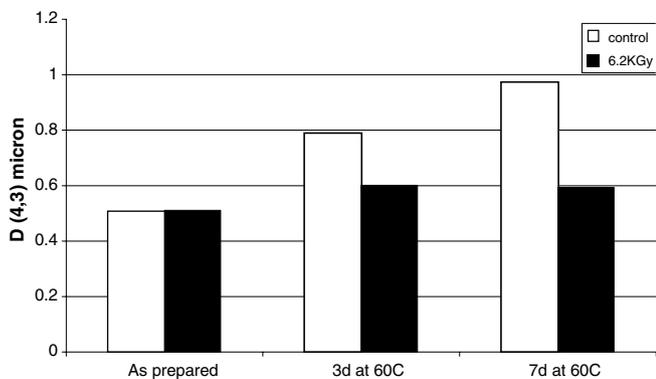


Fig. 4. Volume weight mean (D4,3) for initial and accelerated emulsions made using control (5.9×10^6 g/mol) and irradiated samples for 6.2 kGy in the presence of actylene ($M_w = 2.5 \times 10^6$ g/mol).

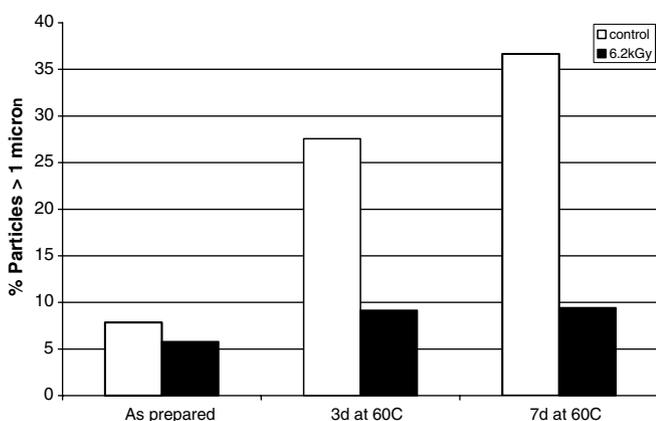


Fig. 5. % Particles greater than 1 micron for initial and accelerated emulsions made using control (5.9×10^6 g/mol) and irradiated samples for 6.2 kGy in the presence of acetylene ($M_w = 2.5 \times 10^6$ g/mol).

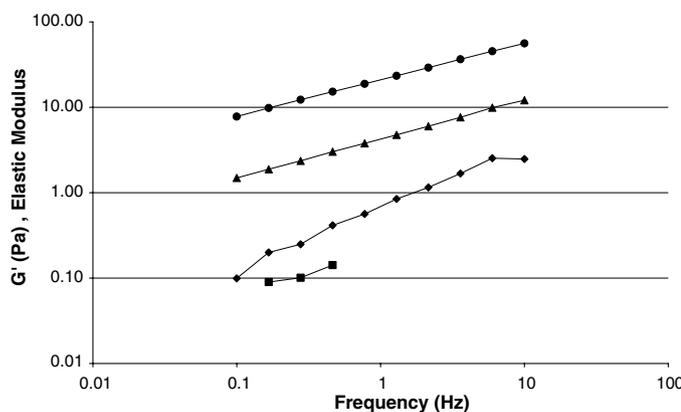


Fig. 6(a). Elastic modulus (G') for control and irradiated samples of spray dried *A. senegal*. 30% w/w in H_2O containing 0.005% w/v NaN_3 as a preservative. (■, control; ◆, 6.2 kGy; ▲, 10.6 kGy; ●, 26.5 kGy.)

increased AGP content and molecular weight of the irradiated sample (Table 1).

The rheological changes induced by the irradiation process were also investigated using small deformation oscillation measurements to monitor the viscoelastic properties.

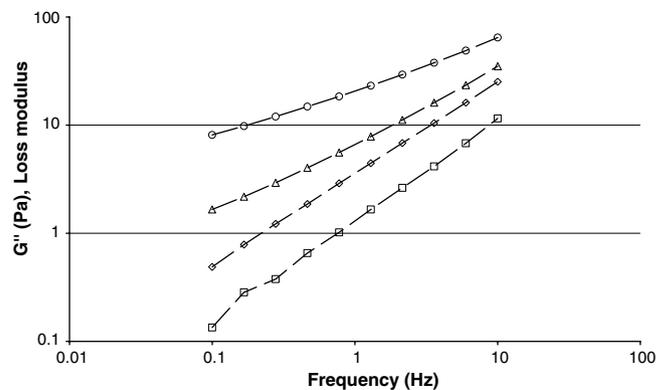


Fig. 6(b). Loss modulus (G'') for control and irradiated *A. senegal* in the presence of actylene. (30% w/w in H_2O containing 0.005% w/v NaN_3 as a preservative. (□, control; ◇, 6.2 kGy; △, 10.6 kGy; ○, 26.5 kGy.)

Figs. 6(a) and 6(b) give the elastic (G') and loss (G'') moduli for control and irradiated samples. The measurement at 30% (w/w) solutions of gum arabic indicates that initially the test material is not sufficiently structured to allow accurate values of G' to be determined for the entire range of frequencies (Fig. 6(a)). However, upon irradiation the value of G' increases with increasing the radiation dose and follows the molecular weight increase. The increase is >100-fold at low frequency. The loss modulus (Fig. 6(b)) shows that initially for the starting material $G'' > G'$ over the entire range of oscillation frequencies and is typical of diluted solution behaviour, when the molecules cannot entangle and produce a network structure during oscillatory stress. Upon treatment and as a consequence of increasing the AGP fraction and hydrogel formation the solution becomes more structured and moves to concentrated solution behaviour (cross over between G' and G''). Here the molecules are capable of entanglement and therefore show elastic response.

Another example to illustrate the effect of the gas mediated process is given here on pectins. Pectin is a complex polysaccharide which has a variety of uses as a thickening, gelling and stabilising agent, particularly in the food industry. It is found in the cell walls of almost all higher plants and it is mainly extracted from the peels of citrus and apple pomace, and from sugar beet pulp [22]. The extracted pectin has varying degree of gelling strength (viscoelasticity) depending on the nature and quality of the starting material. The gel strength is often controlled by blending one or more batches with sufficient sugar to give certain performance required for the application. Pectin is composed of (1–4) linked α -D-galacturonic acid residues in either the free acid (salt) or methyl ester form [23]. The extent of methyl esterification on the galacturonic acid backbone leads to what is known as high methoxyl (HM) pectins (degree of esterification $\geq 50\%$) and low methoxyl (LM) pectins (degree of esterification $< 50\%$).

The changes induced by the radiation process on the viscoelastic properties of three commercial pectin samples (namely LM, HM and sugar beet) are given in Table 2.

Table 2
Elastic modulus (G'), loss modulus (G'') and dynamic viscosity at 0.1 Hz for control and irradiated pectin

Sample	Dose (kGy)	G' (Pa) at 0.1 Hz	G'' (Pa) at 0.1 Hz	Dynamic viscosity (Pa s)
HM pectin	0	2.20	13	20.69
	2.1	8.39	19.45	30.97
	4.9	35.89	35.99	57.29
	6.2	55.94	43.74	69.61
	15.2	6.66	23.13	36.82
Sugar beet pectin	0	3.23	9.88	15.73
	2.1	6.56	12.85	20.45
	4.9	13.18	31.59	50.28
	6.2	11.7	19.97	31.79
	15.2	1.03	4.899	7.797
LM pectin	0	1.44	11.95	19.02
	2.4	2.71	13.75	21.88
	4.7	5.79	18.05	28.73
	6.8	10.34	23.38	37.21
	15.4	11.83	17.95	28.57

Irradiation was carried out in the presence of acetylene.

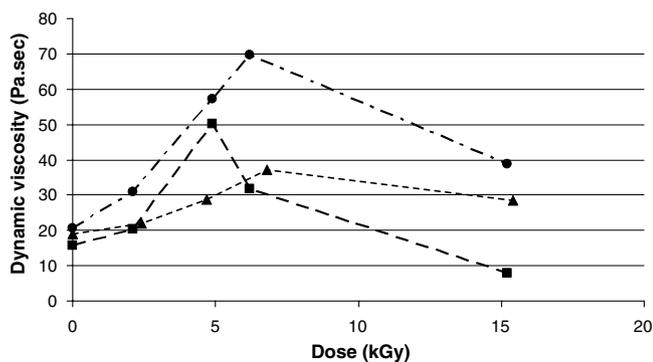


Fig. 7. Dynamic viscosity as a function of radiation dose for (●) HM pectin (Lot AMD 782); (▲) LM pectin (Lot No. TS P6361) and (■) sugar beet pectin (Lot G33213). At 7.5% w/w in water containing 0.005% NaN_3 .

The data given in Table 2 is further illustrated in Fig. 7, a plot of the dynamic viscosity as function of irradiation dose to show the maximum increase in viscosity is dependent on the type of pectin. The latter demonstrate the increase of the viscoelastic parameters of the three pectin types to a maximum followed by hydrogel formation and thus a reduction in viscosity. Thus, the desired product can be achieved by selecting the optimum dose for either increase the viscosity or hydrogel formation.

3.2. Protein system

Previously the radiation modification using the gas mediated process of gelatin was described [18]. Gelatin is derived from the parent collagen by processes that destroy the secondary and higher structures with varying degrees of hydrolysis of the polypeptide backbone. Collagen is major fibrillar protein in the extracellular matrix and in connective tissues such as skin, bone, teeth, cartilage, tendons and ligament. Collagen in the solid state and in the presence of acetylene was subjected to γ -radiation doses of 8

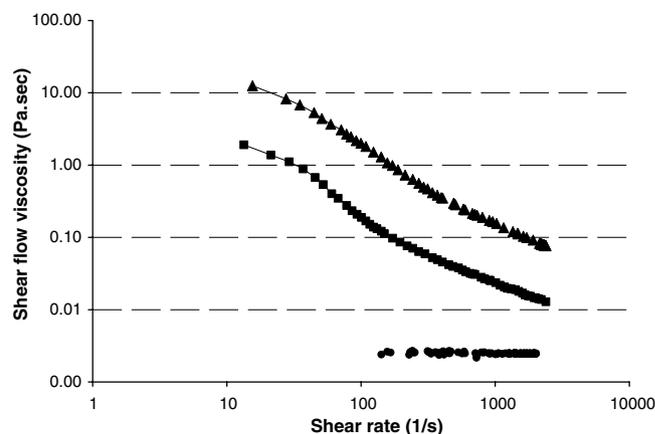


Fig. 8. Shear flow viscosity as a function of shear rate of control and irradiated collagen in the presence of acetylene. Measurements were performed at 2% w/v in 0.1 M NaCl at 25 °C. (●) control; (■) 8 kGy; (▲) 16 kGy.

and 16 kGy. The shear flow viscosity, at room temperature, is shown in Fig. 8 as a function of shear rates for 2% solutions of the control and irradiated collagen. The viscosity of the control was very close to that of water and showed Newtonian behaviour. The viscosity increased with the radiation dose up to 16 kGy. The high molecular weight material produced after 8 kGy was completely soluble with 100 times greater viscosity than the control and further increase up to 1000-fold was observed after 16 kGy. The results shown in Fig. 8 demonstrate that by selecting the optimum dose the molecular weight for collagen can be increased and retaining water solubility. Thereafter at higher doses hydrogel can be formed and the amounts controlled.

4. Conclusion

Irradiation in the solid state and in the presence of acetylene allows systematic modification of polysaccharides and proteins. An example of the irradiation process was given here for a natural exudate gum, *A. senegal*. Its role as an emulsifier is achieved as a consequence of its amphiphilic character due to the presence of protein and polysaccharide moieties. The protein moieties are hydrophobic and strongly adsorb on to the surface of oil droplets, whilst the polysaccharide chains are hydrophilic and extend out into the solution, preventing droplet flocculation and coalescence through electrostatic and steric repulsion forces [24]. The arabinogalactan protein (AGP) fraction is responsible for the emulsifying properties of GA. The results given above demonstrated that the weight average molecular weight can be increased and more importantly the proportion of the high molecular weight arabinogalactan-protein complex (AGP) can also be increased and controlled. Thus, as mentioned above, by controlling the proportion of this fraction the emulsification performance can also be increased. Similar approach was also applied to wide range of hydrocolloids such as low and high

methoxy pectin, sugar beet pectin. The doses of radiation used are very low and up to 6 kGy can more than treble the molecular weight. For comparison the usual sterilisation dose is 25–32 kGy.

The overall rheological parameters too can be controlled. Viscosity increases accompany the molecular weight changes and shear networks too are formed. As the molecular weight reaches that beyond which it is possible to have a complete water soluble system, a gel is formed. This is reflected in the oscillation and shear flow experiments and the gel can be visibly observed also. This product has a great capacity for water absorption. The process allows the production of tailor-made and reproducible materials with enhanced functionality.

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References

- [1] O.H.M. Idris, P.A. Williams, G.O. Phillips, *Food Hydrocolloid* 12 (1998) 379.
- [2] E.A. Balazs and Leshchiner, US Patent No. 4,636,524 (1987).
- [3] Y. Liu, X.Z. Shu, S.D. Gray, G.D. Prestwich, *J. Biomed. Mater. Res. A* 68A (2003) 142.
- [4] C.T. Laurent, K. Hellsing, B. Gelotte, *Acta Chem. Scan.* 18 (1964) 274.
- [5] N. Yui, J. Nihira, T. Okano, Y. Sakurai, *J. Cont. Release* 25 (1993) 133.
- [6] S.K. Hahn, R. Ohri, C.M. Giachelli, *Biotechnol. Bioproc. Eng.* 10 (2005) 125.
- [7] E.A. Balazs, J.L. Denlinger, *J. Rheumatol.* 20 (1993) 3.
- [8] E.A. Balazs, in: D. Miller, R. Stegmann (Eds.), *Sodium hyaluronate and Viscosurgery*, Wiley, New York, 1983, p. 5.
- [9] X.Z. Shu, Y. Liu, F. Palumbo, Y. Luo, G.D. Prestwich, *Biomaterials* 25 (2005) 1339.
- [10] A. Charlesby, in: Farhataziz, A.J. Rogers (Eds.), *Radiat. Chem.*, VCH Publishers, New York, 1987, p. 454, ISBN 0-89573-127-4.
- [11] G.O. Phillips, in: T.C. Laurent (Ed.), *The Chemistry, Biology, and Medical Applications of Hyaluronan and its Derivatives*, Portland Press, London and Miami, 1998, p. 93.
- [12] S.M. Blake, D.J. Deeble, G.O. Phillip, T.A. Du Plessis, *Food Hydrocolloid* 2 (1988) 407.
- [13] R.A. Wach, H. Mitomo, N. Nagasawa, F. Yoshii, *Radiat. Phys. Chem.* 68 (2003) 771.
- [14] T. Katayama, M. Nakauma, S. Todoriki, G.O. Phillips, M. Tada, *Food Hydrocolloid* 20 (2006) 983.
- [15] A. Singh, J. Silverman, *Radiation Processing of Polymers*, Hanser Publishers, New York, 1992.
- [16] G.O. Phillips, T.A. Du Plessis, S. Al-Assaf, and P.A. Williams, US Patent 6,610,810 B2 (2003).
- [17] S. Al-Assaf, G.O. Phillips, P.A. Williams, *Food Hydrocolloid* 19 (2005) 647.
- [18] S. Al-Assaf, G.O. Phillips, P.A. Williams, *Food Hydrocolloid* 20 (2006) 369.
- [19] Prof. J.K. Thomas, Personal Communication (2006).
- [20] A.M. Islam, G.O. Phillips, A. Slijivo, M.J. Snowden, P.A. Williams, *Food Hydrocolloid* 11 (1997) 357.
- [21] S. Al-Assaf, T. Katayama, G.O. Phillips, Y. Sasaki, P.A. Williams, *J. Jpn.* 208 (2003) 771.
- [22] G.A. Morris, T.J. Foster, S.E. Harding, *Food Hydrocolloids* 14 (2000) 227.
- [23] S.K. Sharma, A. Liptay, M. Le Maguer, *Food Res. Internat.* 30 (1998) 543.
- [24] R.C. Randall, G.O. Phillips, P.A. Williams, *Food Hydrocolloids* 2 (1988) 131.