RADIATION PROTECTION ON POLYSACCHARIDE SOLUTIONS AND GELS

by

SEDIGHEALSHARIEH BAZAFKAN

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Department of Biological Sciences

University of Salford

Salford M5 4WT

England

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IN THE NAME OF GOD, THE COMPASSIONATE, THE MERCIFUL

DECLARATION

The work embodied in this thesis was carried out in the Department of Biological Sciences at The University of Salford, under the supervision of Dr John S Moore. This work has not been submitted for any other degree.

	J. Bazafkan
Supervisor	Candidate

To: My husband, Khosrow, Barani

My son Mehdi, my daughter

Yosra and my dear Parents

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May God bless everyone.

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ABSTRACT

Abstract

The effects of ionizing radiation on solutions of (1) sodium alginate, (2) alginate gels (wet and dry), (3) xanthan gum, (4), locust bean gum (LBG) and (5) carboxymethyl cellulose (CMC), have been investigated.

Problems arise in the radiation sterilization of these gums (either as a solution or gel) due to reduction in their viscosity and the gel strength, caused by the high doses (25kGy) commonly used for the purpose of sterilization.

The alginates used here were manugel DMB, manugel DPB and manucol DMF. The irradiations were carried out using ^{60}Co-γ-source and the gels and solutions were analysed by measurement of their apparent viscosities using a Brookfield viscometer L.V.T.

The data shows that 2% solutions of the sodium salt of the three alginates used here are all pseudoplastic.

There is a rapid decrease in viscosity of solutions irradiated up to a dose of 0.5kGy, and the initial rate of viscosity decrease is unaffected by the presence or absence of air. Inclusion of mannitol at high concentration (15%) could, at least partially, protect the alginate solutions degradation by scavenging ·OH radicals.

The possibility of using ^{60}Co-γ-radiation to sterilize alginate gels (wet gels) was also studied. Gels of this type have potential use of wound care. Those prepared here could bend easily. Irradiated to 25 kGy, the gels readily crack by becoming more brittle, are easily squashed and lose water. Inclusion of mannitol improved the quality of the gels and again indicates

its protective role in these systems.

When alginate solutions containing mannitol and the gelling agents δ-gluconolactone and calcium orthophosphate were irradiated separately and then mixed, gels were formed, indicating that pre-sterilization of the components by irradiation is a feasible method of preparation of sterile gels.

Gels that were concentrated (dry) by water evaporation were more stable to radiation (25 kGy). The gels that had the greatest capability to take up saline and to be manipulated most easily (both before and after irradiation) were those that contained initially 2% alginate and 5% mannitol, and dried to a quarter of the original weight (ie. the gel now contained 8% alginate and 20% mannitol). These gels were clear and pliable and after irradiation to 30 kGy remain stable in saline for up to 24 hours.

The effect of ionizing radiation on xanthan and LBG solutions are also studied. The initial studies indicated that these solutions were also pseudoplastic. Irradiation of xanthan gum solutions caused a rapid initial decrease in apparent viscosity. t-Butanol had some protective effect on xanthan-LBG and xanthan-NaCl solutions. Addition of mannitol (20%) to xanthan gum solutions again only partially protects the solutions. The solutions with highest apparent viscosity were those prepared by mixing equal volumes of solutions of LBG 1%, mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) and solutions of xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}). The viscosity of this solution falls from 300,000 cps to 250,000 (shear rate $0.07s^{-1}$), after irradiation to 25 kGy. Therefore, whereas xanthan gum solutions containing readily depolymerized by irradiation, very high viscosity irradiated xanthan solutions containing thickening agents (LBG) and radiation protectors (mannitol -

ascorbic acid) can be prepared. Solutions of CMC are also pseudoplastic. Irradiation to 25 kGy resulted in a decrease in the apparent viscosity of solutions of CMC/mannitol/ascorbic acid from ~ 180,000cps to 8,000cps, whereas for solutions of CMC alone and for CMC/mannitol solutions the viscosity was less than 500cps. This further illustrates the protective effect of ascorbic acid as was observed for xanthan solutions and also suggests that mannitol radicals cause depolymerization of CMC.

CHAPTER ONE

GENERAL

INTRODUCTION

Radiation Chemistry

Radiation chemistry is that branch of chemistry which is concerned with the chemical effects produced by the absorption of high energy from ionizing radiation. The ionizing radiation may be either electromagnetic radiation of short wavelength (between 10-100nm)[1], i.e. with an energy greater between approximately 10-100kev, for example X-rays, γ-rays or particulate radiation, electrons, β-particles, α-particles, protons and fission fragments.

The absorption of light of longer wavelength, by molecules (i.e. photochemistry), may take place in the ultra-violet and visible, infrared or microwave regions leading to electronic, vibrational and rotational energy changes respectively. In terms of photochemistry, however, the ultra-violet and visible regions of the electromagnetic spectrum are of the most interest, because it is here that absorption causes the excitation of the electrons of the molecules which are responsible for chemical binding and the possibility of chemical changes. Thus absorption of radiation of ultra-violet and visible wavelengths leads primarily only to excited states with discrete energies. These energies are much less than the energy of the particles and photons in radiation chemistry.

The principle characteristic of high-energy radiation is that it causes ionization in all materials. This is the major distinction between radiation chemistry and photochemistry, so that radiation chemistry may be regarded as an extension of photochemistry. Radiation chemistry is different from radiochemistry, the latter being the study of the radioactive elements.

The various kinds of emissions from radioactive substances were first observed by Roentgen in (1895)[2]. He indicated that the three different types of radiation (α, β and γ-rays) are

positively charged, negatively charged and electrically neutral respectively, γ-rays are the most penetrating.

In 1896, after Roentgens[2] discovery of X-rays, Henry Bequerel[3] discovered radioactivity. He found that the radioactive element uranium, emits a penetrating form of radiation which caused a blackening of a photographic plate. In 1901 Becquerel[4], published one of the first papers on radiation chemistry containing observations of the chemical effects produced by ionizing radiation. Further studied was the formation of ozone from oxygen[5], and most important of all the decomposition of water by radium[5-10]. Ramsay and Soddy[10], showed that water is quantitatively decomposed into a mixture of hydrogen and oxygen.

Bragg[11], calculated that the number of molecules of water decomposed was approximately equal to the number of ions that are produced in air by the radiation. Many of the high-energy particle accelerators developed for nuclear research have been used to study radiation-chemical problems. However, at present, cobalt-60 is the most widely used source of γ-rays which is used for chemical studies in industry, medicine and research.

The Interaction of Ionizing Radiation and Matter:

An understanding of the mechanism of interaction of radiation with matter is essential to considerations of the chemical and physical effects produced by such radiation. Ionizing radiation produces a nonhomogeneous mixture of ions, radicals and various excited species in the material, the fate of which depends on the density of the matter (absorber) and the nature of ionizing radiation. The high-energy electromagnetic radiations lose energy as it passes through each unit thickness of absorbing material.

The three main processes by which photons lose their energy are:

1. **Compton Scattering:**

In Compton scattering, the main process by which γ-ray photons lose part of their energy, is by ejecting electrons, which results in the formation of excited or ionized molecules. However, not all of the incident photons will interact with matter, the remainder of the energy can produce further scattering or be involved in photoelectric absorption (Figure 1).

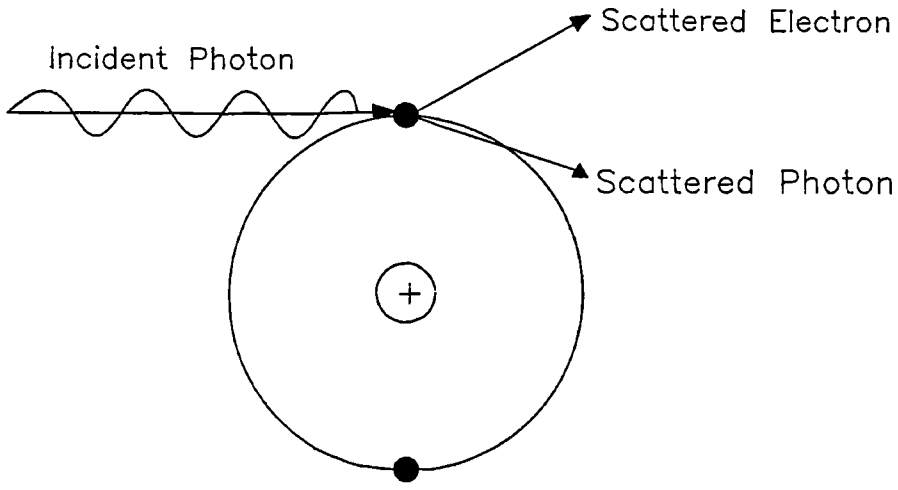


Figure 1 Compton Scattering

2 **Photoelectric Process:**

The main process by which low-energy photons (x-rays and γ-rays) are absorbed is by the photoelectric process. In this type of interaction all the energy of the incident photon is absorbed and is used to eject an electron usually from one of the inner atomic shell. It is then replaced by an electron dropping from an outer shell, with the liberation of energy (Figure 2).

The kinetic energy of the ejected electron is the difference between the energy of the incident radiation and the binding energy of the electron in the atom.

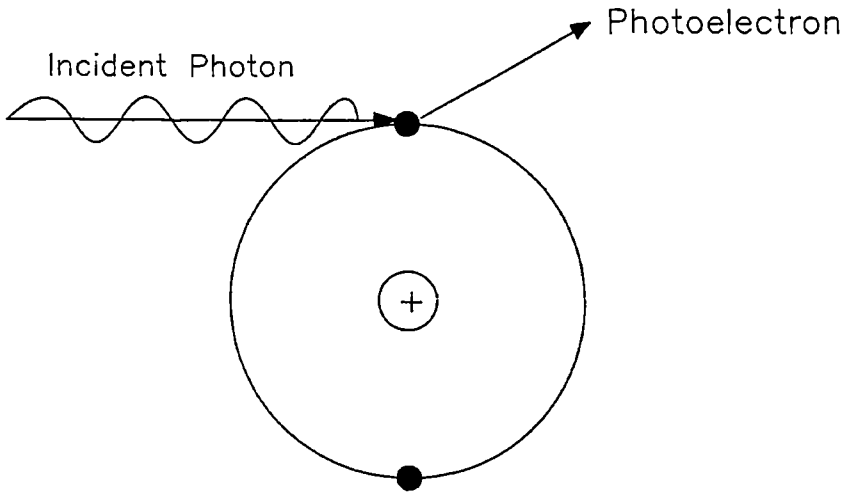


Figure 2 The photoelectric effect

3 Pair Production:

Pair production involves complete absorption of the incident photon, by the electromagnetic field of the nucleus. This in turn is converted into an electron (e^-) and positron (e^+) ion pair, (Figure 3). The electrons and positrons are energetic and lose their energy by causing ionization and excitation.

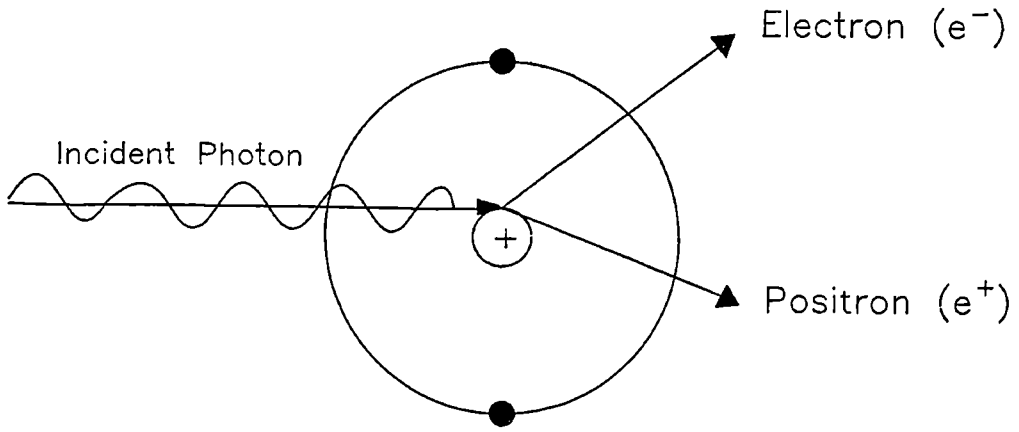


Figure 3. Pair production

The positron is ultimately destroyed by combining with an electron, usually to give two photons each of energy 0.51Mev (annihilation radiation), Figure 4.

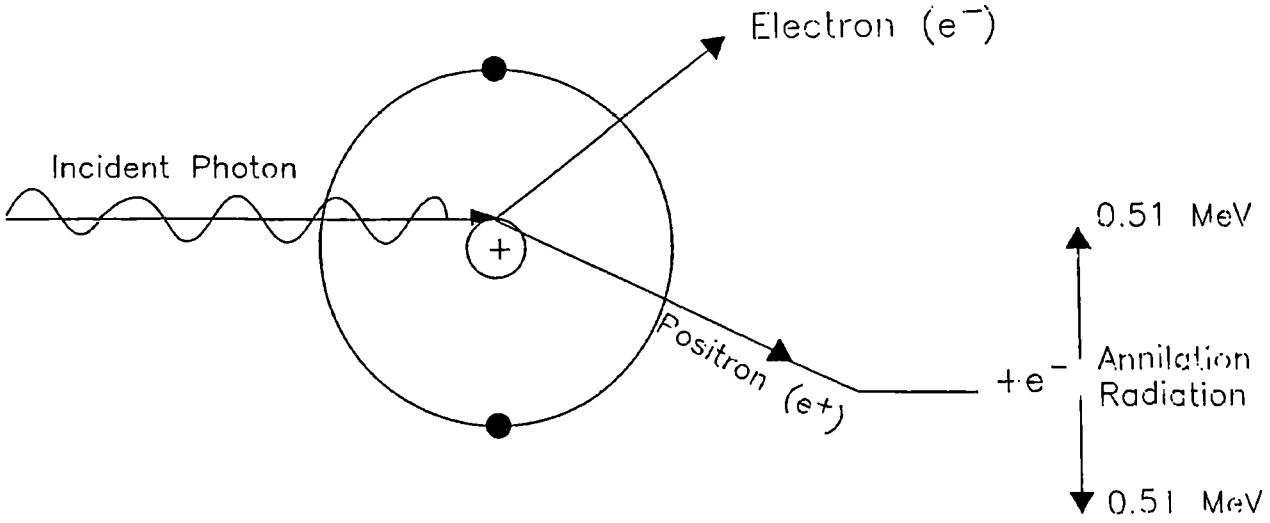


Figure 4 **Kinetic energy of electron, positron, (pair production) resulting of Annihilation radiation.**

As a result of absorption of energy from ionizing radiation the main chemical effect is the formation of fast electrons, which may then cause further excitation and ionization on the medium.

For secondary electrons with γ-rays of energy relatively smaller than 100 ev, the ionization and excitation events are produced inhomogenously along the track of the incident photon. The energy is deposited in small packages called spurs (Figure 5). These spurs can have different sizes. The ions and radical species formed in spurs are very reactive and in their initial inhomogeneous distribution may recombine to form molecular products, or diffuse into the bulk solution[12-21].

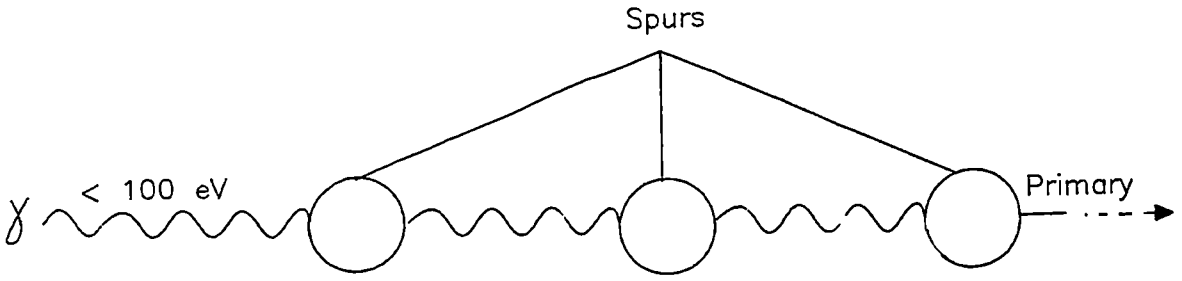


Figure 5 Schematic presentation of the distribution of energy disposition

Towards the end of a track, if the energy of the secondary electrons decreases they are not energetic enough to react and therefore form large spurs called "blobs"[13,22].

The rate of energy loss is generally expressed in terms of the "linear energy transfer" or LET. The units values are usually kilo electron volts per micron (kev μ^{-1}) of path. γ- and x-rays transfer all their energy to the medium through secondary electrons having a wide range of energies and LET. Moreover, much of the ionization and excitation caused by fast electrons of medium energy (e.g.10kev) is produced in δ-ray tracks and clusters, which are branched off from the main path of this particle.

By 1946 it was established that two types of processes occurred when ionizing radiation interact with molecules, namely those interactions that cause direct ionization and those that are indirectly ionizing. Direct action is the interaction of radiation with molecules resulting in chemical changes. Indirect action is the reaction between solute molecules and "activated" solvent molecules (ions, radicals, or excited states). The concept of indirect action resulted from studies of radiation-induced chemical reactions in aqueous solution[23]. However, indirect effects can arise as a result of either ionic or free radical reactions. In fact most indirect action with solutes in aqueous solutions was attributed to the interaction of $H^{\cdot}$, $^{\cdot}OH$ and $\bar{e}_{aq}$ radicals, formed during the radiolysis of water.

Effects of Radiation in Aqueous Systems

Water is a constituent of all living systems and consequently the effects of ionizing radiation on water attracted attention many years ago[1,24-41].

The radiolysis of dilute aqueous solutions indicates that the energy input into the system causes ionization and excitation[42-45] and produces free radicals, ions and molecular products derived from water and dissolved material. These so-called primary products are formed within about 10^{-9}s. after the passage of the ionizing radiation and are the species which bring about the ultimate permanent chemical changes in aqueous systems[46-51].

The formation of free radicals and molecular products is summarized by the following reaction:

$$H_2O \xrightarrow{\sim\sim} H^{\cdot} + {}^{\cdot}OH + \bar{e}_{aq} + H_2 + H_2O_2 + H_3O^+$$

Three different free radicals are formed namely the hydroxyl radical (·OH), the solvated (hydrated) electron ($\bar{e}_{aq}$) and the hydrogen atom (H·). The stable products are molecular hydrogen and hydrogen peroxide.

The ·OH and H_2O_2 are oxidizing products and $\bar{e}_{aq}$, H· and H_2 are the reducing components. The free radicals may react with a variety of dissolved substances and cause chemical changes.

Recombination reactions involving H·, ·OH and $\bar{e}_{aq}$ result in the formation of the molecular products H_2 and H_2O_2.

$H^{\cdot} + H^{\cdot} \rightarrow H_2$ $\quad k = 1 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$

$^{\cdot}OH + {}^{\cdot}OH \rightarrow H_2O_2$ $\quad k = 6 \times 10^{9}\ dm^3\ mol^{-1}\ s^{-1}$

$\bar{e}_{aq} + \bar{e}_{aq} \rightarrow H_2 + 2OH^-$ $\quad k = 5 \times 10^{9}\ dm^3\ mol^{-1}\ s^{-1}$

A considerable part of the radicals formed in the spur are converted to water, protons and hydroxide ions, which neutralize one another[52-56].

$H^{\cdot} + {}^{\cdot}OH \rightarrow H_2O$ $\quad k = 2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$

$\bar{e}_{aq} + {}^{\cdot}OH \rightarrow OH^-$ $\quad k = 3 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$

$H^+ + OH^- \rightarrow H_2O$

Buxton[54] indicated that a considerable number of the radicals that escaped the spur can be interrupted by radical scavengers.

The hydrated electron is more stable than free electrons. It is the strong reducing agent and reacts rapidly with oxygen and H_3O^+.

$\bar{e}_{aq} + O_2 \rightarrow O_2^{\cdot -}$ (superoxide radical) $\quad k = 2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$

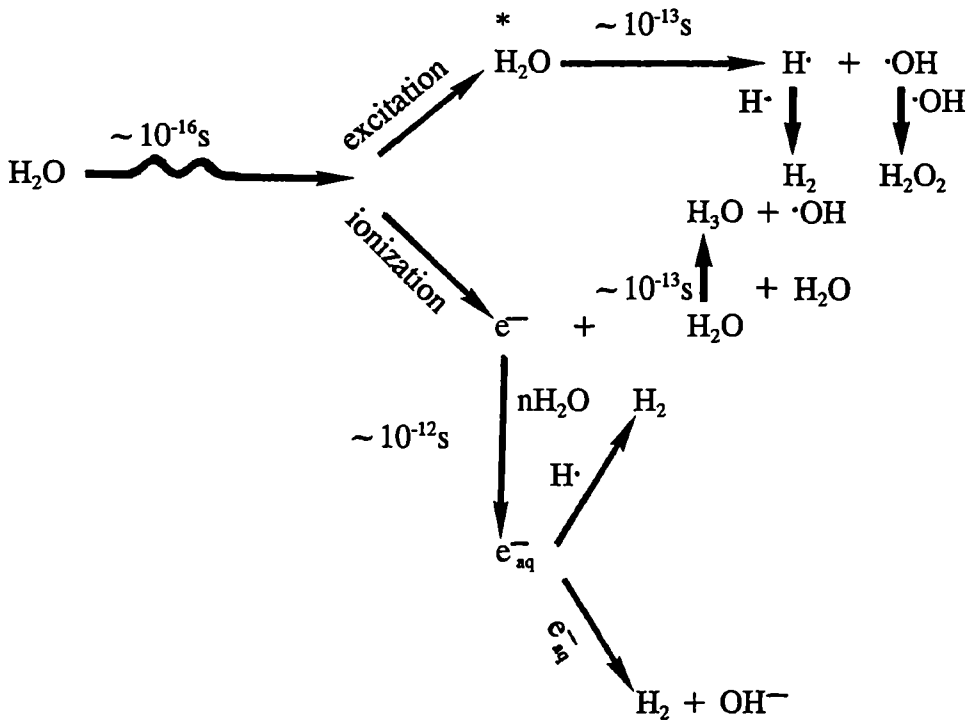
$\bar{e}_{aq} + H_3O^+ \rightarrow H_2O + H^{\cdot}$ (hydrogen atom) $\quad k = 2.2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$

Until 1962, most radiation chemical reactions were attributed to reaction of $H^{\cdot}$ radicals[1,25,26]. Since then it has subsequently been shown that $\bar{e}_{aq}$ behaves like a $H^{\cdot}$ radical in many reactions and that $\bar{e}_{aq}$ can be converted into $H^{\cdot}$ by its reaction with H^+.

$\bar{e}_{aq} + H^+ \rightarrow H^{\cdot}$ $\quad k = 2.2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$

Then in 1962 and 1963, Hart and Boag[56,57] identified the hydrated electron in deaerated water using pulse radiolysis.

The radiolysis reactions leading to the primary products of water are summarized as below:



In order to provide a quantitative basis for radiation induced chemical yield, a unit G-value was first introduced by Burton[58]. The G-value is the number of molecules changed (damaged or destroyed) or of new molecules formed per 100ev of absorbed energy.

$$G = \frac{\text{Number of species produced or disappearing}}{\text{100ev absorbed}}$$

One of the official units of absorbed dose was the rad which was defined in 1953. It is equivalent as:

100 ergs (absorbed) per g of material

$\equiv \quad 10^{-2}\ Jkg^{-1}$

The unit which has now been used is the gray (Gy)

$1Gy \equiv 1Jkg^{-1}$

$\equiv$ 100 rads

$\equiv \quad 6.2 \times 10^{15}\ evg^{-1}$

In SI units: the equivalent of 1G unit is 1.0364×10^{-7} mol material changed per joule energy absorbed.

Conversion from krads to Gy is obtained by:

1krad = 10Gy

Table I: Yield (G-values) of the primary radical and molecular products of γ-irradiated water at neutral pH[59].

Products	G-values
$\bar{e}_{aq}$	2.65
$H^{\cdot}$	0.6
$^{\cdot}OH$	2.7
H_2	0.45
H_2O_2	0.67

Since e^-_{aq} and H are interconvertible, depending on pH, (acid solution):

$$e^-_{aq} + H^+ \rightleftharpoons H\cdot$$

the reducing radicals are often treated as a unit, ie. Gred = ($G e^-_{aq}$ + GH) which gives a G value of 3.2.

Methods for the production of the hydrated electron have been reviewed by many investigators[60-62], and it is mode of reaction with many compounds has been reviewed[63].

The Effect of Scavengers on Radical Yields

1 Concentration:

Radical yields are dependent on solute concentration and increase with increasing concentration. At relatively high concentrations (above 1 mol dm^{-3}), the indirect effects of radiation may merge into direct action and chain reactions[64]. In dilute solutions however i.e. at concentrations $\leq$0.1mol dm^{-3}, indirect action generally occurs. An example of the increasing concentration on the radiation inactivation of L-ascorbic acid in aqueous solution is shown in Figure 6[65]. A greater percentage decomposition occurs at the lower concentration.

As the concentration of the solute increases, reaction of the primary radicals with the solute decreases and increased direct effects are observed.

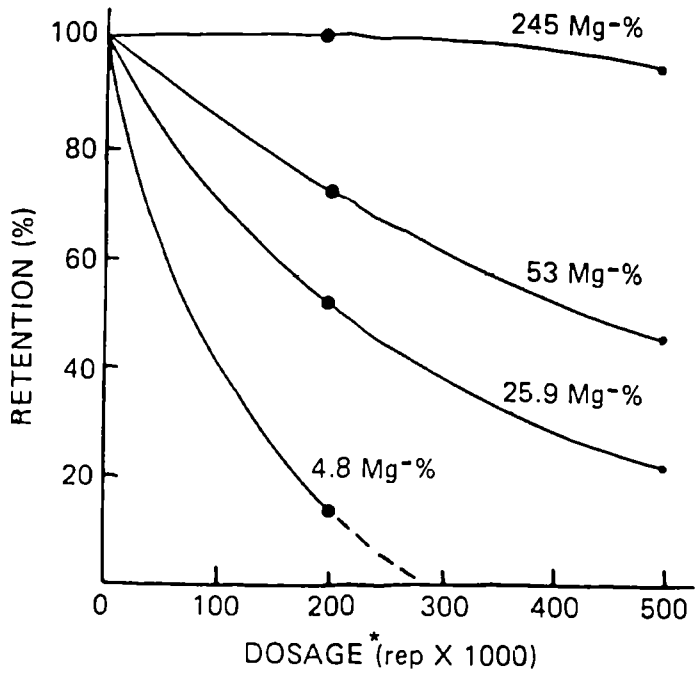


Figure 6: Effect of 3-Mev electron radiation on different concentrations of L-ascorbic acid[65]. *(Roentgen equivalent physical)

2 **Effects of Addition of Second Solute:**

Addition of a second solute into the solution can result in competition with the first solute for the radiolytic products (·OH, H· and $\bar{e}_{aq}$). The greater the ability of the second solute to scavenge radicals, the less the damage will be to the first. This is named the "protection effect"[66]. The efficiency of scavenging a given radical depends on the scavenger concentration and on the rate constant for this reaction with free radical concerned. The concentration should usually be at least 10^{-4} mol dm^{-3}. There is some effect of pH on the primary free-radical yield (some $\bar{e}_{aq}$ can be converted into H· at low pH), but this is not drastic[67]. Foods generally have a number of components and irradiation of moist foods yield numerous radiolytic products, in low concentration by either direct or indirect action.

Scavengers react with the free radicals and effectively "intercept" or protect the other solutes present. Some large complex molecules, containing groups such as SH and NH_2 provide the most effective protection. This observation led to the development of several protective agents[1,68]. Selective free radical scavenging has been used very extensively, particularly for the measurement of the yield of primary radicals in irradiated water[25,26,59,69]. Some other commonly used scavengers are:

Oxygen:

In a variety of systems including biological systems, radiation damage is enhanced by the presence of oxygen. It is an effective scavenger of the primary reducing species $H^{\cdot}$ and $\bar{e}_{aq}$, even at low concentration, although the yield of $^{\cdot}OH$ radicals remains unchanged.

$$O_2 + H^{\cdot} \rightarrow HO^{\cdot}_2 \qquad k = 2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}\ [70]$$

$$O_2 + \bar{e}_{aq} \rightarrow O_2^{\cdot -} \qquad k = 2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}\ [55]$$

The reducing species are therefore rapidly scavenged to produce the perhydroxyl radical ($HO^{\cdot}_2$) and superoxide radical ($O_2^{\cdot -}$). Since the concentration of oxygen in air saturated solutions is about 260μmol dm^{-3}, it competes effectively for $\bar{e}_{aq}$ and $H^{\cdot}$ with most solutes.

The oxygen effect is likely to be influenced by the concentration and type of solute molecules, the nature of the suspending media, pH, and the presence of impurities (eg. SH compounds[71,72]. In systems containing many organic molecules, the presence of oxygen causes a damage in the pattern of degradation and alters their G values[1,25-27]. Ozone, a very powerful oxidant, is readily formed from oxygen and sometimes can be smelled in the room housing the $^{60}Co\gamma$ source.

Nitrous Oxide

Nitrous oxide is the favourite and most common selective scavenger of hydrated electrons, because the overall process is relatively uncomplicated. In N_2O saturated solution, $\bar{e}_{aq}$ are quantitatively converted into $^{\bullet}OH$, thus effectively doubling the $^{\bullet}OH$ yield[73,74].

$$\bar{e}_{aq} + N_2O \xrightarrow{+H_2O} {}^{\bullet}OH + N_2 + OH^- \qquad k = 9.1 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}[75]$$

This system therefore has the advantage that more $^{\bullet}OH$ radicals are produced (which are the main oxidising radicals), and can attack and contribute in any damaging processes (eg. killing of bacteria).

In the presence of N_2O, G($^{\bullet}OH$) is 5.5, which accounts for ~90% of all primary radical yield, the remaining ~10% are $H^{\bullet}$atoms[75].

In solutions saturated with N_2 or O_2 the G-value of $^{\bullet}OH$ (G = 2.8) is less than that in N_2O. The nitrogen that is also produced is not a radical scavenger and does not produce radicals.

Hydrogen

The primary oxidizing radical ($^{\bullet}OH$) are converted then into $H^{\bullet}$atoms, in the presence of hydrogen at high pressures of about 100 atmospheres. The $^{\bullet}OH$ radicals react with H_2 as follows:

$$^{\bullet}OH + H_2 \rightarrow H^{\bullet} + H_2O \qquad k = 3 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

In alkaline solution, H·atoms are converted to hydrated electrons:-

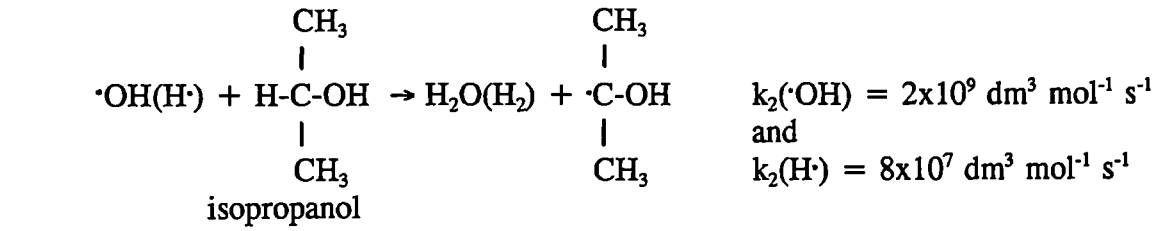
$$H\cdot + OH^- \rightarrow e^-_{aq} + H_2O \quad k = 1.8 \times 10^7\ dm^3\ mol^{-1}s^{-1}$$

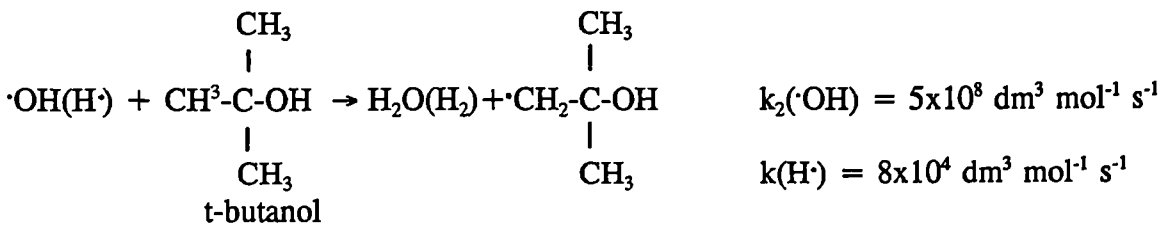
under these conditions the only surviving transient radical is e^-_{aq}. In acid solution, hydrated electrons are rapidly converted to hydrogen atoms.

$$H^+ + e^-_{aq} \rightarrow H\cdot \quad k = 2.2 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$$

Some other common scavengers for ·OH radicals are formate, alcohols and iodide and some other common e^-_{aq} scavengers are, chloracetic acid, acetone and chloroform.

For example ·OH radicals and H· atoms are effectively scavenged by isopropanol and t-butanol.





In formate solution, the following reactions occur:-

$$\cdot OH + HCOO^- \rightarrow H_2O + CO^{\cdot}_2{}^- \quad k_2 = 4x10^8\ dm^3\ mol^{-1}\ s^{-1}$$

$$H^{\cdot} + HCOO^{-} \rightarrow H_2 + CO_2^{\cdot -} \qquad k_2 = 2.5 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

In the case of chloracetic acid the H· atoms react mainly by H· abstraction, whereas the e_{aq}^- react via dissociative electron capture.

$$H^{\cdot} + ClCH_2COOH \rightarrow H_2 + {}^{\cdot}CHClCOOH$$

$$e_{aq}^- + ClCH_2COOH \rightarrow Cl^- + {}^{\cdot}CH_2COOH$$

Effect of Ionizing Radiation on Carbohydrates in Aqueous Solutions

Carbohydrates are an important group of naturally occurring organic compounds widely distributed in animals and plants. They act as a store or reservoir from which the plant or animal may derive energy for the maintenance of cellular activities, or, form an integral and essential component of food. Carbohydrates generally occur in nature associated with water and with other naturally occurring macromolecules, such as a structural proteins or as the sugar backbone in DNA and RNA. Starch is the major storage carbohydrate in plants, and glycogen is the only carbohydrate stored in animals.

The major structural polysaccharide in plants is the neutral polysaccharide cellulose. Anionic polysaccharides also fulfil this function, both in plants and animals, for example, alginate and carrageenan occur in seaweeds and the glycosaminoglycans, for example, hyaluronic acid and the chondroitin sulphates are found in the animal connective tissues, or as a matrix in which mucopolysaccharide, protein and water are associated.

The radiation chemistry of carbohydrates and polysaccharides, has been studied since about 1912[76-80], following studies on model compounds such as alcohols and hydroxy acids.

Physical changes that occur following irradiation of low molecular weight carbohydrates include changes in pH, optical rotation, viscosity and absorption spectra[79,81,82].

Later studies continued into investigations of the chemical changes that occur in carbohydrates and the changes produced in simple model compounds such as alcohols[1].

Various methods have been used to investigate the primary processes that occur during radiolysis of carbohydrates, both in aqueous solutions and the solid state, for example, pulse radiolysis, using optical systems for detection of the unstable radicals produced, has been used by many groups for solutions of carbohydrates[83-87], and electron spin resonance (esr) is commonly used to identify the radicals produced in solid carbohydrates[88].

Many studies have also been carried out into the nature of the permanent products that are commonly produced, e.g. compounds containing the functional groups (C = O, $-CH_2-$ COOH, -CHO) are often formed. The products of irradiation can be analysed, using the suitable separation methods. Chromatography, using paper[89], partition[90,91], ion-exchange[92,93], gel[94-97], gas-liquid chromatography (GLC)[98-101], combined gas chromatography-mass spectroscopy (GC-MS[92,99,102-105], and high-performance liquid chromatography (HPLC), have been widely used. Paper chromatography has been extensively used for the determination of the concentration of certain carbohydrates in solution[89], and for following the kinetics of degradation and the formation of radiolysis products[106-113]. GLC has been used for the study of low-molecular-weight products from irradiation of polysaccharides such as starch[114]. Furthermore the quantification for radiation-chemical yields can also be determined using GLC.

Some of the primary free radical reactions which are ultimately responsible for the decomposition of the carbohydrate have been investigated using ESR spectroscopy and pulse radiolysis. These investigations provide the basis of the interpretations of the mechanism proposed for concepts derived formation of products.

Information regarding the overall changes that occur in irradiated polysaccharides can be obtained from changes in optical activity or viscosity measurements. However, such observations do not give information on the nature of the chemical changes that occur. Most studies in this area have been carried out on mono and disaccharides in aqueous solution and reaction mechanisms for the formation of the products have been proposed[115-122].

Other investigations were concerned with the influence of different parameters, such as concentration, dose rate and the presence or absence of oxygen on the product distribution. Others deal with the radiolytic degradation of carbohydrates which are of particular interest for the understanding of radiation-induced changes in foods and related substances.

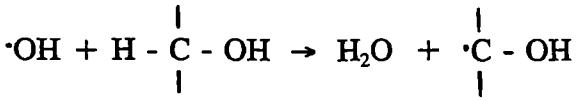
Here, attention will be directed to the mechanisms of degradation of carbohydrate systems, irradiated in aqueous solution.

Primary Stages of Radiolysis Processes

Irradiation of aqueous solutions causes the formation of the reactive species, e^-_{aq}, ·OH and H· atoms and the molecular products, H_2, H_2O_2.

$$H_2O \xrightarrow{\sim\!\!\sim} {\cdot}OH,\ e^-_{aq},\ H{\cdot},\ H_2,\ H_2O_2$$

Solvated electrons react slowly with carbohydrates[123], whereas ·OH, radicals and H· atoms react quite readily. Both ·OH and H· abstract carbon-bound hydrogen atoms from the carbohydrate.



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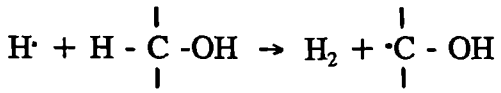
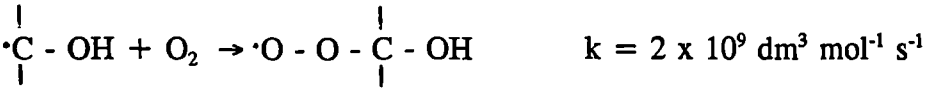


Molecular oxygen adds to primary carbohydrate radicals, to give peroxyl radicals[124].



Oxidative degradation is therefore the most general consequence of the action of ionizing radiations on carbohydrates. Low molecular weight sugars in aqueous solution may undergo oxidative degradation, partly due to the direct action of the radiation and partly due to interaction with the radiolytic products of water mainly ·OH radicals. The mechanisms, for formation of products due to oxidation or the terminal carbons are readily understood. However irradiation generally, induces the formation of acids and ring opening.

Monosaccharides

D-Glucose is a typical monosaccharide, the ·OH radicals can abstract almost at random any of the carbon-bound hydrogens, due to their high reactivity. There is a slight preference for an abstraction at $C_{(1)}$ since this is the most loosely bound hydrogen and also at $C_{(6)}$ which carries two hydrogens[125]. The formation of acidic products from ^{60}Co-γ-irradiated of glucose has been widely reported[126-127]. The main products of the acidic fraction are showed

in Figure 7 all of which contain the complete C_6-skeleton of the irradiated carbohydrate. The reactions formed from the glycosyl radical at the C-1 position is as follows[127].

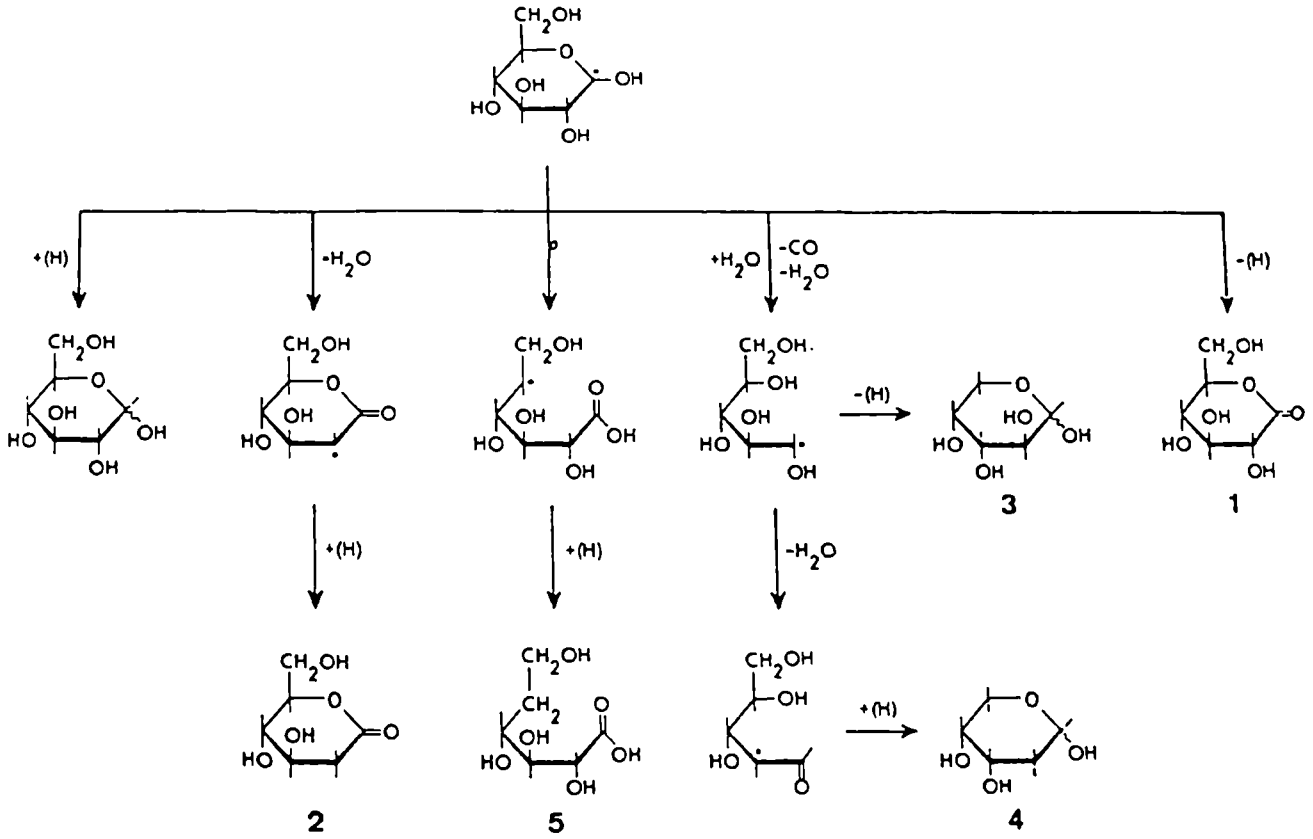


Figure 7: Product formation after radical attack at C-1 of glucose. 1, Gluconic acid; 2, 2-deoxy-gluconic acid; 3, arabinose; 4,2-deoxy-ribose; 5,5-deoxy-gluconic acid.

Dizdaroglu, *et al*[128], showed that the yield of 2-deoxy-gluconic acid decreased at increasing glucose concentration. Furthermore the yield of gluconic acid increased, while the total G-value for acids (G=1.06) is independent of the glucose concentration.

Preliminary comparison of different monosaccharides revealed that their radiation-chemical susceptibility did not depend upon the character of sugar[106], but was influenced by changes in their concentration and radiation conditions (Table II).

Table II: The decomposition yields of some monosaccharides.

Monosaccharide	Irradiation conditions [atmosphere/ concentration (mol dm^{-3})]				References
	in vacuo/ 0.05	inert gas/ 0.01, 0.05	Oxygen/ 0.05	Nitrous oxide/ 0.01	
D- glucose	3.5	2.4, 3.1	3.5	5.6	106,128,131
D-galactose	-	2.5, 3.0	-	-	106
D-mannose	3.5	2.8, 3.1	3.5	-	106,132,131
D-ribose	-	- -	3.2[a]	-	133
2-deoxy-D-ribose	-	- -	-	6.5[b]	137

a) C = 3×10^{-3} mol dm^{-3}

b) C = 8×10^{-3} mol dm^{-3}

The chemical changes that occur in irradiated monosaccharides can be almost entirely attributed to reaction of the ·OH radical, since the yields of decomposition increase in N_2O compared with irradiation in vacuum. Further evidence comes from pulse radiolysis and the use of competing radical scavengers[129].

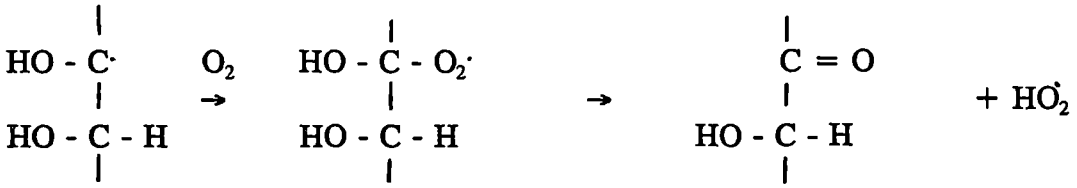
Pulse radiolysis experiments showed that the degradation can proceed via chemically and kinetically different routes depending on the glucose concentration[130]. The higher the concentration of solution, the greater is yield of decomposition of monosaccharides (Table III).

Table III: Concentration dependence of the yield of decomposition of monosaccharides.

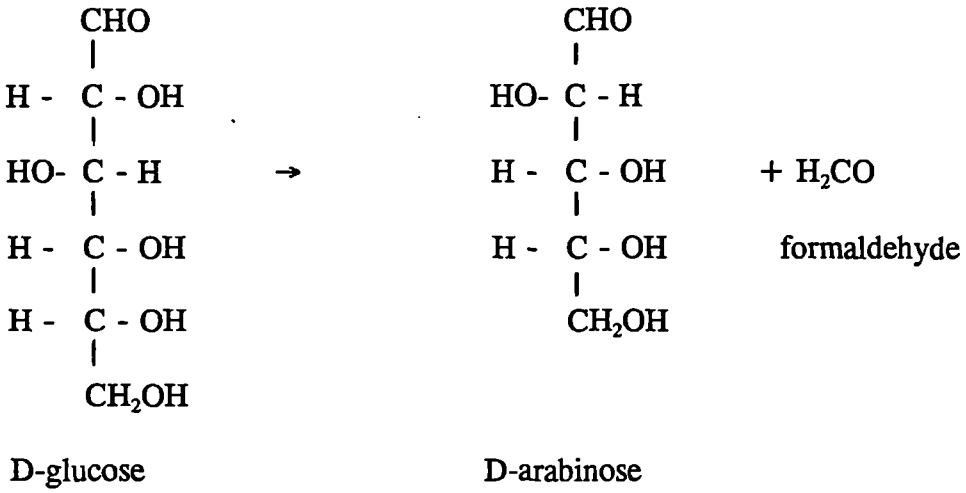
Monosaccharide	Yield of decomposition					References
	0.0005*	0.005	0.01	0.05	0.1	
D- glucose	1.5	2.5	2.5	3.1	3.5	106
D-mannose	-	2.3	2.8	3.1	3.7	130

*C = mol dm^{-3}

The presence of oxygen increased the yield of acids and keto acids.



In certain cases, however formaldehyde and arabinose arise from, eg. glucose irradiated in the presence of oxygen.



D-glucose D-arabinose

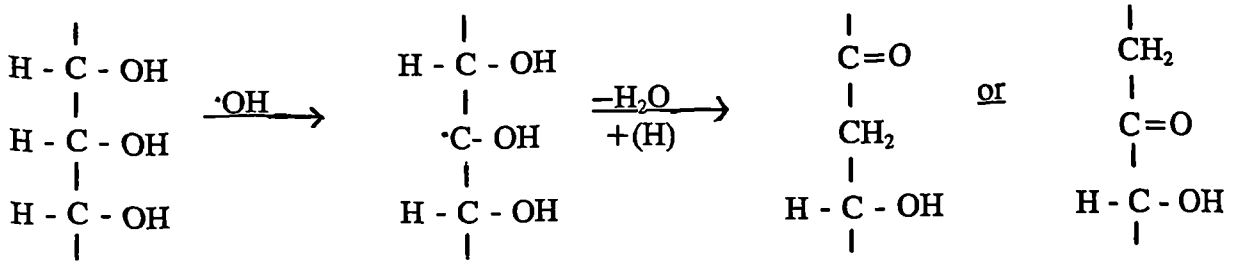
Scission of the carbon-chain accompanies oxidation of monosaccharides and leads to the

formation of acids, formaldehyde and keto acids. The extent of C-C bond scission was found to be lower after irradiation *in vacuo* compared with that formed in the presence of oxygen.

Irradiation of 0.05 mol dm^{-3} solutions of glucose and mannose *in vacuo* gave two-carbon fragment yields of 0.85 and 0.95[131], the yields of three-carbon fragments amounted to 0.8 and 0.5 respectively. Irradiation in oxygen of 0.05 mol dm^{-3} solutions of glucose[131], mannose[132] and ribose[133], gave yields of two-carbon fragments of 0.8, 0.7 and 1.3 and that of three-carbon fragments from ribose was 0.8. Arabinose, erythrose, tetradialdose, erythrulose, glyceraldehyde and dihydroxyacetone were detected among the products of glucose degradation in 0.01 mol dm^{-3} glucose in the presence of oxygen[134]. The extent of this process depends largely upon the irradiation conditions and solute concentration.

A considerable part of the radiation-induced transformation of monosaccharides are lead to deoxy- and deoxy-keto compounds as final products. The yield of deoxy sugars is pH-dependent. For example for evacuated solutions of D-glucose (5.5×10^{-2} mol dm^{-3}), the following G-values were obtained: 0.1, 0.26, 0.52 at pH 1, 7-9 and 11-12 respectively[135]. Therefore irradiation at higher pH increase the yield of deoxy compounds.

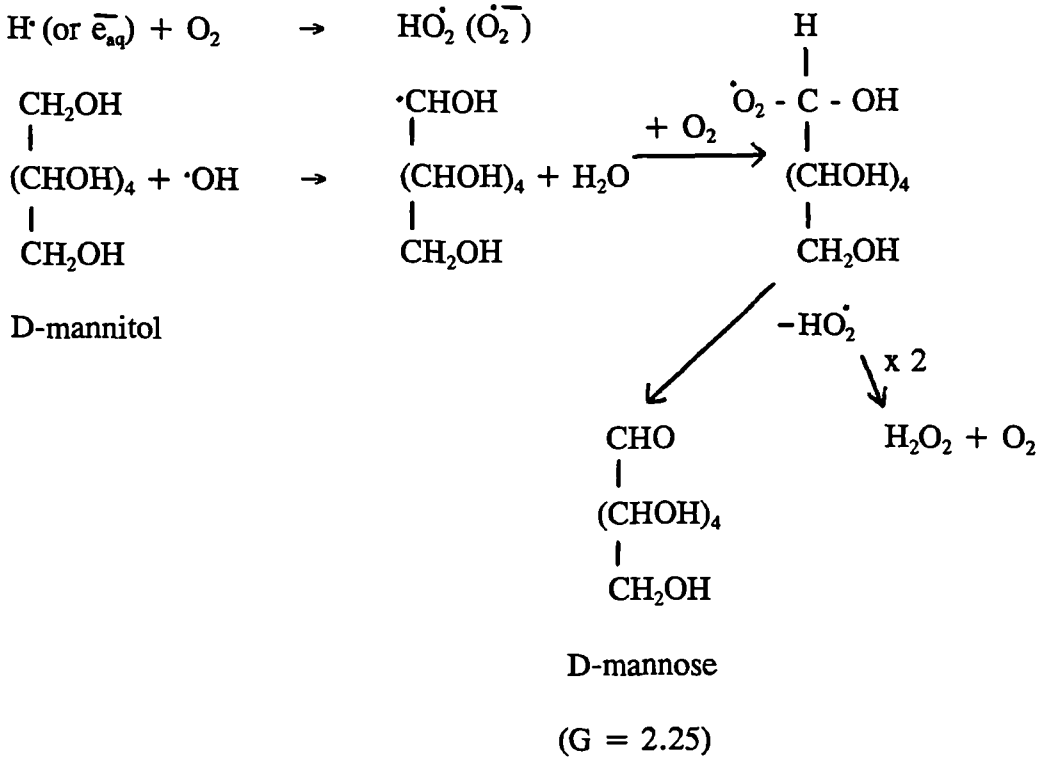
Deoxyketo-compounds are formed, generally in higher yield than deoxy-compounds, G-values of 0.24, 0.4 and 0.25 were obtained for the radiolysis of N_2O-saturated solutions of glucose, galactose and mannose[136]. The mechanism of the formation of these products is proposed to be as follows:



The structures of several deoxy- and deoxy-keto-compound radiolysis products from 2-deoxy-D-ribose[137] and D-glucose[138] (N_2O- saturated solutions) and from D-glucose and D-fructose[139-141] (in N_2) have been established.

Malondialdehyde (MDA), a common product in monosaccharide radiolysis, also results from carbon-carbon bond scission. It was shown to be a primary radiolysis product and its yield increases linearly with increasing dose in vacuum, N_2, O_2 and N_2O[135,142]. The yield of MDA greatly depends upon pH and was formed to be 0.04, 0.06 and 0.08 for neutral and acidic, 0.01 mol dm^{-3} solutions of galactose, mannose and glucose and 0.86, 0.59, 0.53, 0.53, 0.86, 0.95 and 1.09 for 0.05 mol dm^{-3} solutions (pH13) of arabinose, xylose, ribose, galactose, mannose, glucose and fructose[135]. The detailed mechanism of MDA formation of pentoses, disaccharides and polyhydroxyalcohols, has been studied using pulse radiolysis[143].

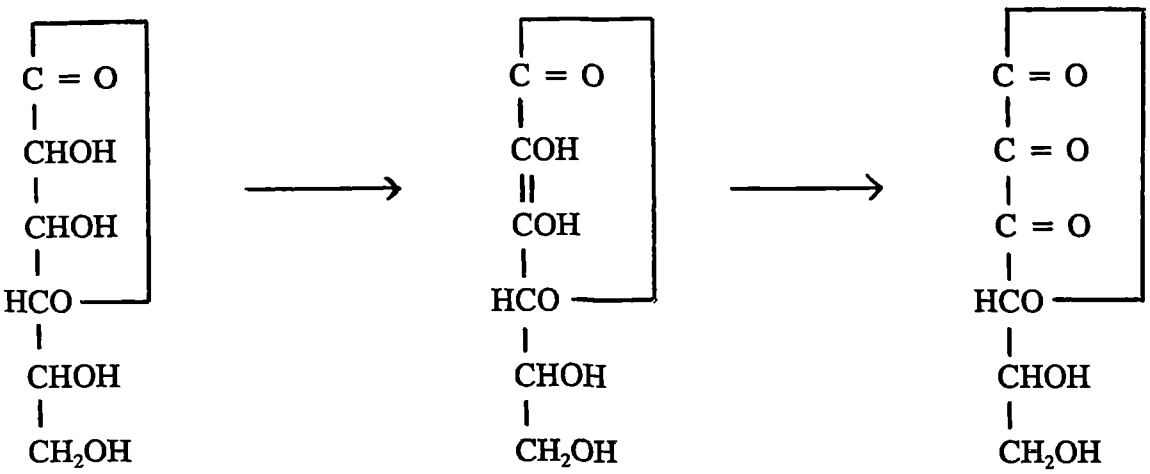
During radiolysis of low molecular weight sugars in aqueous solution, differences in end products which are formed relate to their initial molecular structure. Irradiation of oxygenated D-mannitol solutions[144,145] produced D-mannose (G = 2.25) and H_2O_2 (G = 3.0). The yield of mannose approximates to GOH and that of H_2O_2 to $GH_2O_2 + G\bar{e}_{aq} + GH$ so that the mechanism can be represented as:



Subsequent reactions of the primary radical, the addition of the oxygen and decomposition of the peroxy radical are well published[146].

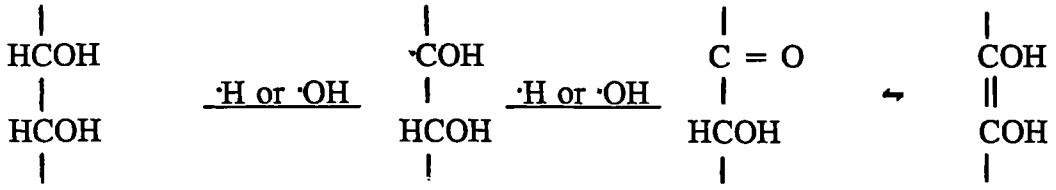
Studies of the radiolysis of aqueous solutions of the preservative (antioxidant) ascorbic acid, were carried out in the presence of oxygen[147,152]. Investigation of the radiolysis of this vitamin is important in relation to the radiation preservation of foods. Ascorbic acid is very labile towards the action of radiation.

Coleby showed that when lactone solutions are irradiated with x-ray, ^{60}Co0γ-rays or fast electrons under vacuum, the lactones are converted into the corresponding ascorbic acids.



Lactone Ascorbic acid (AH_2) Dehydro-Ascorbate (A)

The route to the ascorbic acid from the lactone may involve abstraction of a hydrogen atom at C-2 or C-3, followed by enolization.



Ascorbic acid (AH_2) is readily oxidized to dehydro-ascorbic acid (A) during irradiation of its aqueous solutions. In acidified aerated solutions the consumption of ascorbic acid (-G ascorbate) and oxygen (-G O_2) are 7.8, 7.49 respectively. The reactions can be described as:

$$AH_2 + {}^{\cdot}OH \rightarrow AH^{\cdot} + H_2O$$

$$AH_2 + HO^{\cdot}_2 \rightarrow AH^{\cdot} + H_2O_2 \qquad k = 3.3 \times 10^3 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

$$AH^{\cdot} + O_2 \rightarrow AHO^{\cdot}_2$$

$$2AHO^{\cdot}_2 \rightarrow 2A + H_2O_2 + O_2$$

Radiolytically generated $HO^{\cdot}_2$ radicals oxidize the ascorbate (to $AH^{\cdot}$) and further oxygen

addition may occur to form the ascorbate peroxy radical $AHO_2^{\bullet}$. This explains the higher value for (-G O_2). The decomposition yield does not change at concentration higher $> 10^{-3}$ mol dm^{-3} and is equal to 4.8, 3.4 and 1.8 irradiation in oxygen, nitrogen and carbon dioxide respectively[149-150]. Addition of CO_2 and 0.02 mol dm^{-3} formic acid increase its radiation stability. At higher doses, the main radiolysis product [dehydro-ascorbate (A), G = 4.8[149]], undergoes rapid decomposition, oxalic acid and carbon dioxide were identified as the secondary products of ascorbic acid radiolysis[150].

It was suggested[149], that the high yield of decomposition of ascorbic acid (AH_2) in the presence of oxygen is connected with the interaction of all the water radiolysis products in the formation of the primary radicals, these then accept O_2 to produce a peroxyradical which yields the final product (A). In the absence of oxygen the same final products were formed by disproportionation of the primary radicals. At neutral pH ascorbic acid is attacked by $\bar{e}_{aq}$ and $\dot{O}H$. For doses up to 5kGy, only small losses of ascorbic acid have been observed in fruits and in vegetables, such as potatoes[154], onions[153-155] and tomatoes[156].

Glucuronic acid is a major component of numerous biopolymers of plant and animal origin, eg. alginic and hyaluronic acids, so that studies of its radiolysis are of considerable interest. Irradiation of 5 x 10^{-4} mol dm^{-3} solutions of glucuronic acid (GA) in N_2O and 10^{-2} mol dm^{-3} solution in O_2, resulted in decomposition yields of 1.3 and 3.6 respectively[119].

Disaccharides

The predominant effect of radiation on disaccharides is scission of the glycosidic bond. The radiation susceptibility of the glycosidic bond of different disaccharides such as cellobiose, maltose, lactose, gentiobiose, melibiose, trehalose and sucrose has been studied[157]. Table

IV summarises data for 10^{-2} mol dm^{-3} solutions of the disaccharides. A detailed study was carried out by C.Von Sonntag *et al*, of N_2O-saturated solutions of cellobiose[158]. Twenty one monomeric products were identified and their G-values measured, (Table V).

The products were analysed using combined gas chromatography-mass spectrometry and they estimated that about one-third of the products formed by reaction·OH radicals, result from scission of the glycosidic bond. The presence of O_2 reduces this effect[159]. Hydrolysis at the glycosidic bond also occurs during irradiation of sucrose in aqueous solution, and acids are produced[159-160]. Yield-dose curves showed that D-glucose and D-fructose are primary products of the γ-irradiation of dilute, aqueous sucrose solution, in the presence of oxygen, together with smaller amounts of D-arabino-hexosulose, D-gluconic acid and D-glucuronic acid, D-arabino-hexulosonic acid, D-arabinose and two- and three-carbon aldehydic fragments arise by secondary processes[161,162].

The primary process leading simultaneously to D-gluconic acid, D-arabinohexosulose, D-glucose and D-fructose may be accounted for by two types of oxidative scission of the disaccharide linkage. The former leads to D-fructose and D-gluconic acid with initial G-values of 1.5 to 1.6 and 0.4 respectively, and the later to D-glucose and D-arabino-hexulosonic acid with G-values of 1.5 to 1.6 and 0.6 respectively.

The same two models of cleavage of the glycosidic linkage were found also for lactose[163] and maltose[164]. A series of oxidized disaccharide derivatives were obtained upon irradiation of 0.03 mol dm^{-3} maltose solution in the presence of O_2[164].

MDA is formed with a yield of 0.1 in neutral solution[135], and in considerably higher yield

Table IV: Radiolysis of Disaccharides

Disaccharide	Irradiation conditions	Yield of decomposition	Yield of monosacharide	References
Cellobiose	N_2O	6.5	1.5(23)*,2.1(33)	101,103,104
	N_2	3.1	0.8(26)	101
	O_2	2.8	1.0(35)	101
Lactose	N_2O	4.0	Glucose 1.1(28)	
			Galactose 0.6(14)	103,105
	N_2	2.3	Glucose 0.7(30)	
			Galactose 0.4(19)	105
Gentiobiose	N_2O	-	1.6	103
	N_2	4.0	1.2(30)	101
Melibiose	N_2O	-	Glucose 1.15	103
			Galactose 0.5	
Trehalose	N_2O	-	3.5	103
Sucrose	N_2O	-	Glucose 1.6	103
			Fructose 1.1	106
	O_2	4.0	Glucose 1.5(37)	
			Fructose 1.5(37)	
Maltose	N_2O	-	2.0	103
	N_2	3.3	1.0(30)	101
	$O_2(3x10^{-2}M)$	4.0	2.4(60)	107

* Figures in brackets indicate the percentage of monosaccharide formed with respect to total radiolysis products

Table V: **^{60}Co-γ-radiolysis of N_2O-saturated O_2-free aqueous solutions of cellobiose ($10^{-2}M$)[158]**

No	Monomeric Products	Initial G-value
1	Glucose	2.1
2	Gluconic acid	0.70
3	5-keto-glucose	0.05
4	4-keto-glucose	0.07
5	4-deoxy-glucose	0.27
6	5-deoxy-gluconic acid	0.18
7	2-deoxy-gluconic acid	0.13
8	3-deoxy-4-keto-glucose	0.23
9	2-deoxy-5-keto-glucose	0.34
10	4-deoxy-5-keto-glucose	0.14
11	6-deoxy-5-keto-glucose	0.02
12	Arabinose	0.07
13	Ribose	0.015
14	2-deoxy-ribose	0.17
15	3-deoxy-pentulose	0.01
16	Erythrose	0.015
17	Threose	0.015
18	2-deoxy-tetrose	0.01
19	Butanone-(2)-diol(1,4)	0.01
20	Dihydroxyacetone	0.01
21	Carbon monoxide	0.02

in alkaline medium. G(MDA) from 10^{-2} mol dm^{-3} solutions of lactose, maltose and sucrose, (N_2O atmosphere, pH13), were 0.36, 0.44 and 0.88 respectively[135].

Disaccharides, therefore undergo transformations which are common to monosaccharides, namely oxidation, oxidative destruction and formation of deoxy- and deoxy-keto compounds. The radiation induced scission of the glycosidic bond is also characteristic of all disaccharides.

Polysaccharides

The most important effect of irradiation of polysaccharides is scission of glycosidic bond, which is accompanied by a decrease in molecular weight. There are many studies that have been carried out include cellulose[165,166], starch[167-170], agar[171], alginic acids[172-174], various gums[167-172], pectins[172,175,177], glycogen[178], hyaluronic acid[179-182], heparin and keratan sulphate[183,184], chondroitin-4-sulphate[185], inoline[186] and dextran[187-189].

There is some evidence also for dimerization in certain polysacchrides. A small number of breaks or crosslinks can drastically alter the physical properties of polymer. Degradation of polysaccharides is accompanied by a decrease in viscosity[171,172,176,178-180,182,187-196]. In the 'dry' state degradation leads to an increase in solubility and a decrease in fibre strength. These effects have to be taken into account in the use of radiation to sterilize certain medical supplies. Early report on the effects of radiation on carbohydrates have been established by Phillips[197-199], and more recently reviewed by C. Von Sonntag[200].

The chemical changes that occur are similar to those for simple carbohydrates. Degradation is accompanied by the formation of reducing groups[172,181,190-191] (carbonyl) and acid

groups[187,194,195,201]. The presence of oxygen decreases the yield of reducing substances and increase the yield of acid[187,201]. Increasing irradiation dose is accompanied by increase in reducing power, ie. which is an indication of scission of glycosidic linkage and the formation of carbonyl- containing compounds[202-205]. Studies of some polysaccharides under different irradiation conditions revealed that reducing components were primary radiolysis products and produced mainly by the action of ·OH radicals[206-208], comparison of increasing reducing power of irradiated polysaccharides in N_2O and deaerated solution is given in Table VI.

Table VI: The yield of reducing compounds during radiolysis of polysaccharides.

Polysaccharide	Irradiation Conditions			Reference
	O_2	Inert gas	N_2O	
Laminarin	2.9	2.1	3.1	206
Heparin	0.5	0.7	1.6	184
Keratan sulphate	1.3	0.9	1.4	183
Chondroitin-4-sulphate	-	0.4	0.8	185

Oxidative processes leading to acidic products play an important role in the radiolysis of polysaccharides. The chemical nature of the acidic products has not been established as to whether they are polymeric or monomeric. However, D-gluconic, D-glucuronic and other

acids were identified among the products of radiolysis of several glycans such as amylose, dextran and starch[202,203,209]. The yields of acidic components of some polysaccharides in Table VI are shown in Table VII.

Table VII: Yield of acidic components upon radiolysis of polysaccharides

Polysaccharide	Irradiation Conditions			Reference
	O_2	Ar.	N_2O	
Heparin	2.8	2.8	5.0	183
Keratan sulphate	4.7	2.7	5.5	185
Chondroitin-4-sulphate	-	2.5	4.9	183

In a recent study it has been shown that the oxidation processes which occur during radiolysis of oligo-and polysaccharides are accompanied by the formation of deoxy- and deoxy-keto compounds[136,210,211].

Despite the numerous studies that have been carried out on polysaccharide solutions all of the radiolysis processes are still not fully understood. Nevertheless, in general, features of the process have been identified[211,212]. Irradiation of aqueous solutions of polysaccharides results chiefly in depolymerization and, to a lesser extent, the modification of individual units without chain scission leading to the formation of -CO-, -CHO, -COOH and -CH_2- groups.

Low molecular products formed are monosaccharides, disaccharides, deoxysugars, acids and

products of more complex transformation. The main features of polysaccharide radiolysis are therefore similar to those found in monosaccharides and glycosides.

Much interest in the radiation chemistry of carbohydrates arises from the commercial and industrial potential of food processing, stabilization and preservation or to extend their shelf life using ionizing radiation[213].

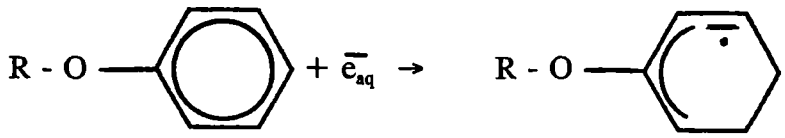
The group of polymers of polysaccharides, such as alginates, carboxymethylcellulose and gums (xanthan gum) have widespread commercial uses, particularly in pharmaceuticals where they are used for formation of gels, films and filaments, cosmetics and food stuffs, where they are used as stabilisers and thickening or gelling agents by increasing the viscosity of food suspensions and thus reduce sedimentation and coagulation. In pharmaceuticals alginates can be applied in the form of fibre or powder which have a marked haemostatic action. They also are gradually absorbed by vascular tissues and therefore can be used as a surgical dressing which can be left in the body[214]. Furthermore injection of a low molecular weight alginate solution can be used at the same time as an antigen, followed by a calcium salt to give a gelled deposit, to increase the response of the organism to antigen[215].

Summary and Conclusions

Summarizing, therefore, it would appear that almost all carbohydrates, when irradiated in aqueous solutions have comparable radiation stability. Their transformations, in aqueous solution, is due to action of H· and ·OH radicals which leads to C-H bonds scission, although interaction with e^-_{aq} in certain instances (carbohydrate with some functional groups such as a free carbonyl, double bond or aromatic residue) cannot be disregarded.

$$R - CH = CH - R' + e^-_{aq} \rightarrow R - \bar{C}H - \dot{C}H - R'$$

$$R - \underset{\underset{O}{\|}}{C} - R' + e^-_{aq} \rightarrow R - \underset{\underset{O^-}{|}}{\dot{C}} - R'$$



On the basis of the similarity of the radiolytic pathways in simple low molecular weight bond sugars and those in more complicated polymeric materials containing these subunits, the final radiolysis products and main processes for the majority of carbohydrates are:

1 $\dot{O}H$ radical is a powerful oxidizing agent and is very reactive with carbohydrates.

$$\dot{O}H + \text{glucose} \rightarrow R\cdot + H_2O \quad k = 10^9 \text{ dm mol}^{-1} \text{ s}^{-1}$$

2 Use of ·OH radical scavengers showed its participation in the radiation induced damage of carbohydrates.

$$\cdot OH + I^- \rightarrow OH^- + I\cdot \quad k = 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$$

3 Oxidation leading to keto-derivatives and acids.

4 Oxidative degradation with C-C bonds scission and formation of oxidized fragments.

5 Formation of deoxy- and deoxy-keto sugars.

6 Formation of dimers and, under certain conditions, polymers.

Irradiation of Food

The world has ample food to feed everybody, and food requirements continue to grow, but, in an environment of scarce resources and limitations on methods of food production and storage. This may be one of the main problems of poverty and hunger. Also it is clear that the available stock of food in the world is unevenly distributed, and large areas in the developing countries experience serious shortages. The situation is made even worse because large quantities of food are lost, after harvesting, due to pests, insects, bacteria, fungi and enzymes which eat, degrade or destroy the crops. Furthermore developing and industrialised countries face an additional problem that of contamination of foods with micro-organisms causing food-borne disease. The incidence of food borne disease has shown a dramatic increase since 1945 and is today one of the most widespread health problems in the world. Therefore, problems of food storage and processing make it necessary to search for effective alternative methods of food preservation. There are a number of very old methods for example, sun-drying, salting and smoking and more modern preservation techniques eg. canning, pasteurizing, deep freezing and vacuum drying have been applied successfully for some time. Chemical preservatives and insecticides also are used to control pests and insects. However, many of the chemicals that were formerly approved for crop storage and quarantine treatment are no longer considered safe because of the potential dangers to humans and the environment. Accordingly, the possibility that these problems can be inhibited by ionising radiation is one of the alternatives for food storage and preservation, provided that it does not adversely affect the wholesomeness of food. However, it was not until the 1950's that the possibility of using ionising radiation, to prolong the lifetime of certain foods, became a serious proposition, when it was shown that irradiation could kill bacteria and retard the sprouting and ripening of fruits and vegetables. It can however, offer new possibilities for increasing the total amount of food available for consumption by avoiding

the losses sustained at present during storage and distribution throughout the world. Furthermore, irradiation treatment should enable the use of chemical preservatives and pesticides to be substantially reduced.

Food irradiation has however, its own advantages and disadvantages. Like all other food preservation technologies proponents often call the technology a method to solve world hunger. Opponents, meantime, often claim that it is dangerous because eating irradiated food or even living near an irradiation facility can lead to cancer. They also think the technique will be misused to make unwholesome food appear fresh. Therefore commercial uses of irradiation techniques are still limited. A possible reason for this reluctance is the feeling that not enough is known about what happens to foods when they are irradiated. One approach has been to analyse irradiated foods, to identify radiation-induced compounds and to estimate their yields. Another approach has been directed at studies of the radiation chemistry of model systems of various degrees of complexity, ranging from pure carbohydrates, proteins, lipids, etc., to multicompound mixtures approaching the composition of food.

The treatment of food by ionising radiation is accepted for specific purposes in several countries, although in other countries the sale of irradiated food for human consumption is prohibited. However domestic commercial interest in food irradiation declined from the early 1970's into the current decade, when they became part of the diets for US astronauts and Soviet cosmonauts, specially bred laboratory animals, and for agricultural and medical purposes, such as patients suffering from AIDS or those requiring bone marrow transplant. Such patients require absolute sterility to reduce the risk of infections from food. Well-defined legislation and appropriate regulations and standards to control the use of irradiation

technology, and to enforce proper public health standards, safety and quality control, are essential. General standards to facilitate international trade in foods and crops processed by irradiation and a code of conduct on food irradiation have been developed under the aegis of World Health Organisaiton (WHO) and the Food and Agriculture Organization (FAO). At present, thirty different kinds of food have been approved for irradiation from application from more than 30 countries, some of which is for general sale and others only for specific uses, eg. agricultural and medical purposes. This list is growing and as progress towards an international agreement on the use of the technology is reached, its commercial application will gain increasing acceptance[216-218]. In the field of medicine, diagnoses and treatment are carried out daily with the aid of radioisotopes. Disposable medical and hygienic products sterilised by irradiation are used regularly in hospitals and clinics all over the world. The United States Food and Drug Administration (USFDA) have adopted several regulations on low-dose irradiation of foods (Code of Federal Regulations, 1988, Fed. Regist. 1986, 1988) and the US Army has maintained an interest in high-dose radiation of meat products.

Ionising radiation changes the texture and nutritional value of food, as well as causing chemical modification. If radiation is to be used successfully this must be avoided. The Advisory Committee on Irradiated and Novel Foods (ACINF) recommended that irradiation of food up to 10kGy with energies up to 5 or 10 Mev should be permitted. However, different applications of food irradiation can be classified by dose level to achieve different aims.

Low-doses up to about 1kGy are used to inhibit sprouting of potatoes and garlic, to control insect infestations and to delay maturity. Medium-doses (Ca. 1 to 10kGy) are used to reduce microbial load, prolong shelf life of products and to reduce the load of nonsporing pathogens

in these products. High-doses (10 to 50kGy) are used to achieve commercial sterilization thus enabling food products to be stored at ambient temperatures with suitable packaging, ie. shelf-stable products. High-dose radiation will eliminate viruses. The different doses used for food irradiation can be summarized as shown in Table VIII[219-220]. Irradiation is a physical method of processing foods, whereby foods are exposed to γ-rays, x-rays or electrons over a limited period of time.

Table VIII: Dose ranges that have been recommended for certain purposes

Process	Approximate Dose Range (kGy)
Inhibition of sprouting	0.05 - 0.15
Delaying of ripening of various fruits	0.2 - 0.5
Insect disinfestation	0.2 - 1.0
Elimination of various parasites	0.03 - 6.0
Shelf-life extension by reduction of microbial load	0.5 - 5.0
Elimination of non-sporing pathogen	3.0 - 10.0
Bacterial sterilization	up to 50.0

X-rays and electrons are generated by appropriate machines, whereas gamma rays are emitted by the radionuclides cobalt 60 and caesium-137. Both of these sources are specifically manufactured for use in the sterilization of medical products and the irradiation of food. At present, almost all radiation facilities in the world use cobalt-60 rather than caesium-137. However, both of these sources are readily available and because of their relatively low energy do not cause the food to become radioactive. Nevertheless, the radiation is highly penetrating and destroys organisms such as bacteria (eg. *Salmonella* and *Clostridium botulinum*). Cell death is due to chemical changes induced by the radiation and strand breaks in certain macro-molecules, such as DNA and RNA. Also, radiation could change the natural process of cell division, or affect hormones that control growth.

Two nuclei, namely oxygen-17 and carbon-14, which are commonly found in food in small quantities, contribute to the natural radioactivity of food but the half-life of oxygen-17 is only approximately 30 seconds. However, because of small quantities of other radionucleids, such as carbon-14, potassium-40 and rubidium-87, food is naturally radioactive; the half life for rubidium is 47 billion years.

Radiation affects the constituents of foodstuff in different ways. The major components of foods are large molecules such as polysaccharides and proteins, but also present are low molecular weight lipids, carbohydrates and vitamins and these are readily decomposed. It is not always possible to extrapolate the data obtained by irradiation of single molecules to that obtained when complex mixtures are irradiated. Differences in their behaviour are observed in certain instances, though the reasons for this are not fully understood. Certainly radiation effects on every food component have not been studied. Food contains some microorganisms such as bacteria, moulds and viruses, that are harmless, though some are

dangerous and food poisoning can occur due to, for example *Clostridium botulinum*. In some instances the radiation affect on the DNA might convert microbes mutant into a more dangerous form. Therefore food irradiation could pose an undetectable (and unacceptable) hazard to the consumer.

The safety of materials consumed by man is of utmost importance, and so it is necessary to ensure that not even the slightest hazard exists from toxic or radioactive substances produced by the radiation. The task of proving this was coordinated by the international project in the field of food irradiation in which WHO participated in an observer capacity.

These projects were periodically controlled and the data obtained were reviewed by the Joint FAO/WHO/IAEA (International Atomic Energy Agency) Expert Committees (JECFI, 1981). The UK Advisory Committee on Irradiated and Novel Foods (ACINF, 1986), concluded that irradiation of any commodity up to an overall average dose of 10kGy, presented no toxicological hazard and no special nutritional or microbiological problems.

The International Committee on Food Microbiology and Hygiene (ICFMH) of the International Union of Microbiological Societies, also, concluded that there was no cause for concern. There was no qualitative difference between the kind of mutations induced by ionizing radiation and that induced by other preservation processes, such as heat treatment or vacuum drying.

However after all the economic, technical and regulatory conditions have been satisfied, the issue of consumer acceptance of irradiated foods remains a fundamental prerequisite for the introduction of the technology. The reluctance of consumers in some countries to accept

irradiated food arises from the fact that anything associated with nuclear energy is considered by many people to involve danger and radioactivity. Furthermore, with the growing preference for fresh foods in the developed countries, anything other than minimal processing and the limited use of additives in foods is increasingly seen as undesirable and possibly harmful to health.

Food irradiation tends to be seen as yet another method of tampering with the natural properties of fresh foods. Even within the food industry, acceptance of the technology has come about only slowly as the benefits of the process became obvious.

Widespread information campaigns are still required for food irradiation to be fully accepted. WHO is concerned that rejection of the process is essentially based on emotional or ideological influences and may hamper its use in those countries which may benefit the most.

Rheology and Viscosity:

It is important to define the above terms, they are often mistaken as referring to the same parameters.

Rheology:

The term "rheology" was first introduced by Bingham and refers to the study of the deformation and flow of materials in a liquid, melt, or solid form in terms of the elasticity, viscosity and plasticity of the material. This definition was accepted when the American Society of Rheology was founded in 1929.

Viscosity:

The term viscosity is derived from the Latin, "viscum", meaning sticky. Therefore viscosity is the degree of "stickiness". However viscosity is a measure of the internal friction of a resistance of the material to flow. This friction becomes apparent when a layer of fluid is made to move in relation to another layer. The greater the friction, the greater force required to cause this movement, which is called "shear". Shearing occurs whenever the fluid is moved or distributed as in pouring, spreading, spraying and mixing.

Newton expressed viscosity as follows:

$$\frac{F}{A} = \eta \frac{dV}{dX}$$

Where η is a constant for a given material and is called its viscosity. The usual unit of measurement is the poise. The SI unit is the Pascal second where 10 poise is equal to Pascal second or (10 poise = Pascal second). The ratio $\frac{F}{A}$ is the force per unit area required to produce the shearing action. It is usually referred to as the shear stress (F) and the unit of measurement is dynes per square centimetre (dynes/cm^2). The velocity gradient $\frac{dV}{dX}$ is the measure of the speed at which the intermediate layers move with respect to each other. It describes the shearing the liquid experience and is thus called the "shear rate" (S). The unit of measurement is the reciprocols/second (sec^{-1}), the general equation then becomes viscosity is equal to shear stress divided by shear rate.

$$\eta = \text{viscosity} = \frac{F}{S} = \frac{\textit{shear stress}}{\textit{shear rate}} \quad \frac{(dynes/cm^2)}{(sec^{-1})}$$

The Nature of Fluids:

Fluids that obey the above equation are described as Newtonian. Newton assumed that all materials have a viscosity that is independent of the shear rate at a given temperature. In other words, twice the force would move the fluid twice as fast. This assumption is ideal but rarely found and is only partly correct. In reality for the rheologist, the majority of materials exhibit complex non-Newtonian behaviour.

Fluids therefore can be categorised into two groups, either Newtonian or non-Newtonian.

Newtonian Fluids:

A Newtonian fluid will give a constant viscosity with variable shear rates, regardless of which viscometer model, spindle or speed used. Examples of Newtonian fluids are silicon oils, thin motor oils, alcohols and water.

Non-Newtonian:

Non-Newtonian fluids are dependent on shear rate. The viscosity measurements of these fluids will therefore change as the shear rate is varied and the measured viscosity is called the "apparent viscosity".

Non-Newtonian fluids can be categorised into two groups: those which are shear rate dependent and those which are time dependent.

1) Shear Rate Dependent:

This type of fluid can also be subclassified into two: pseudoplastic and dilatant.

Pseudoplastic:

In this type of fluid the viscosity decreases with increase in shear rate. These fluids are the most common type of non-Newtonian fluids, e.g. molten polymers, polymer solutions, bread dough and a variety of suspensions, emulsions, dispersions and other structured fluids used as pharmaceuticals and cosmetics.

This type of flow behaviour is also called "shear-thinning".

Dilatant:

In this type of fluid the viscosity increases with an increase in shear rate. Typical examples are moist beach sand, quick sand, polyvinylchloride (PVC) plastisos, aqueous suspensions of penicillin powder, corn starch/water mixtures and clay slurries. Dilatancy is also referred to as "shear-thickening", flow behaviour.

2) Time Dependence:

The effect of time on non-Newtonian fluids leads us also to two more types of sub classes "thixotropic" and "rheopectic". These fluids show a change in viscosity with time under constant shear rate.

Thixotropic:

In this type of fluid the viscosity decreases with time under constant shear rate. Typical examples include emulsion paints, inks, mayonnaise and cough medicines.

Rheopectic:

In this type of fluid the viscosity increases with time under constant shear rate, these fluids are not very common. Some adhesives and custards may exhibit this behaviour.

Plastic:

This type of fluid behaves as a solid under static conditions. A certain amount of force must be put into the system to get the fluid to flow. This force is called the "yield value" or "yield stress". Non drip paints and ketchup are good examples of plastic fluids. Once the yield value is overcome then the fluid can behave as a Newtonian or non-Newtonian fluid (thixotropic, pseudoplastic or dilatant).

There are several reasons why values of the flow properties of various materials are important. They are needed to characterise the state of a substance, e.g. measuring the viscosity of paint in order to characterise the degree of dispersion of a pigment, for food materials, (which may be the raw material, the intermediate or the final product), the flow properties are important in the design of the manufacturing process.

Many fluids formed in biological processes are extremely complex mixtures of polymers and result in the formation of suspensions, emulsion and creams and their rheological properties must invariably be well defined. In this study we concentrate solely on the viscosity of the solutions.

The Effect of Rate of Shear:

As described earlier there may be two anomalous forms of shear rate dependence of viscosity. The viscosity may either increase or decrease with increasing shear rate. The former property is known as dilatancy and it is restricted largely to concentrated suspensions.

The latter is a common feature with solutions of linear polymers and it is known as structural viscosity. It was supposed that structural viscosity was caused by the formation of a loose network of long chain molecules. When subjected to a shear stress this structure breaks and it will cause a decrease of the viscosity (pseudoplastic). Kuhn and Kuhn[221] assume that structural viscosity in dilute solutions cannot be explained by the interaction between the colloid particles and thus must be due to the hydrodynamic properties of the isolated particles. They showed that dilute colloid solutions of easily deformed particles show an almost Newtonian behaviour (i.e. the viscosity is independent of shear stress).

Philippoff and Hess[222] investigated the relationship between shear stress and viscosity for a series of colloidal solutions. They found that viscosity is shear stress dependent. When the shear stress is low, the viscosity of the solutions obeys Newton's law quite well, but when the shear stress is increased beyond a certain limit, the viscosity decreases rapidly and at very high values of shear stress, the viscosity again becomes Newtonian.

It is always advisable to make viscosity measurements at several shear rates in order to detect any rheological behaviour that may have an effect on the processing or use of the sample. Where shear rate values are unknown or not important, a simple plot of viscosity versus revolutions per minute (RPM) will often suffice.

Paints, cosmetics, liquid latex, coating, certain food products and blood in the human circulatory system are good examples of materials that are affected by wide variations in shear rate during processing and use.

The Effect of Temperature:

Temperature can also have an effect on the rheological behaviour of a material. Some materials are sensitive to temperature and could change viscosity, while others are insensitive. For some materials such as motor oils, greases and hotmelt adhesives, consideration of the effect of temperature on viscosity is essential.

The Effect of Time:

For a certain class of fluid the apparent viscosity continues to change as a function of the time for which the particular shear rate is applied. Changes in the viscosity of many materials can occur over time even though the material is not being sheared.

Viscosity Measurement Conditions:

It may be useful to classify fluids according to the various types of non-Newtonian flow properties which they possess. For example, a suspension of rigid particles can exhibit Newtonian flow. Pseudoplasticity or dilatancy depending upon inherent variables, e.g. rate of shear, the type of viscometer model and variables such as spindle speed, temperature and sample preparation technique. All of these variables can have a considerable effect on the value of the viscosity measurement. It is therefore important to be aware of, and to control as far as possible, the environment of any sample to be tested. Knowledge of the nature of the sample whose viscosity is to be measured is very important, e.g. sensitivity of fluids to heat or ageing. Thus, storage conditions and sample preparation techniques must be designed to minimize their effect on subsequent viscosity tests. Thixotropic materials, in particular, are sensitive to prior history as their viscosity will be affected by stirring, mixing, pouring or any other activity which produces shear in the sample.

From these examples, it is apparent that understanding rheology is essential in an industrial environment and is vital in identifying and measuring the desirable properties of new products.

There is no doubt that rheology is a rapidly expanding science. It is also recognised to be a difficult subject. While modern instruments are available to measure materials and produce computerised results, a basic understanding of the underlying theory is needed for interpretation. For example, the subject is inter-disciplinary and most scientists and engineers have to move away from a possibly restricted expertise and develop a broader scientific approach. The theoretician with a background in continuum mechanics needs to develop an appreciation of certain aspects in physical chemistry, statistical mechanics and other disciplines related to microrheological studies to fully appreciate the breadth of present day rheological knowledge.

CHAPTER TWO

INTRODUCTION TO GUMS

Introduction

The sea as well as land has provided man with a source of nourishment and raw materials ever since his development and many of these raw materials, eg. seaweeds, algae or gums, are today essentially the same as they were thousands of years ago.

Stabilizers are widely used in the food industry as an additive because they give a measure of control of the liquid water phase in food products. Most stabilizers are naturally occurring polysaccharides obtained from land and sea plants though some are chemically modified or synthesised. They are used in food products for the purpose of thickening or increasing viscosity, for gelling, suspending and stabilizing. They are also of great importance in the convenience and low calorie food products. Chemically they are polymeric carbohydrates built up of identical repeating units with molecular weights ranging from 2500 to several millions. Because they are of colloidal size they are often referred to as hydrocolloids. They are able to interact with themselves and with other molecules in their environment within the system. In food this system often contains water, sugars, proteins and lipids.

As mentioned above these called stabilizers polysaccharides can be natural or modified polysaccharides. They are sometimes described as gums, the term "gums" arising originally from our use of sticky and viscous plant exudates and seed polysaccharides of high molecular weight. They can be classified as follows:

Natural Gums

These can be separated into three major categories according to the raw materials, source and origin.

(1) Natural Plant Exudates: eg. gum arabic, karaya, tragacantha and ghati.

(2) Plant Seed Gums: eg. guar gum, locust bean gum (L.B.G), tamorind, psyllium seed gum and quince seed gum.

(3) Seaweed Extracts: eg. agar-agar, carrageenan, alginates and furcellaran.

A widely-used gum, xanthan, does not fit into any of these categories, because it is obtained from bacterial sources.

Synthetic or Modified Gums

A number of synthetic gums have been developed, some of which have found extensive use in foods. Several cellulose ethers including sodium carboxymethylcellulose (NaCMC) offer several advantages over natural gums.

The Molecular Structure of Gums

Gums are polymeric compounds built up of identical or related units. The nature of the building units (amino acids, pyranose ring, etc.) is of importance eg. the presence or absence of hydrophillic (eg. OH, NH, COOH), hydrophobic or ionized groups. The actual shape of the building blocks may also be significant.

A second aspect is the nature of the linkage between the units in the polymer which may affect the conformation properties and stability of the material.

Seaweed Extracts

There are four major groups of seaweeds, the algal plants namely Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae) and Cyanophyceae (blue-

green algae). From the commercial point of view only the Rhodophyceae and Phaeophyceae are of significance. They are primarily salt-water plants. The large brown algae are the dominating benthonic algae in cold waters, while the red algae play more significant roles in the warmer seas. They are important commercially because of their polysaccharide content and because they are available in quantities sufficient to support a sizeable industry. Agar and Carrageenan are extracted from various types of the red algae, and algin is derived from the brown seaweeds. Marine algae have been used in food for humans for generations. It has also been used as an animal feed stuff, and in the production of fertilisers, soda water, iodine and other inorganic chemicals.

Alginate (largely the sodium salt form) is a type of polysaccharide which occurs in large quantities in most brown algae. In 1881 while attempting to find a use for the seaweeds, Stanford[223] discovered a colloid that he named algin. He suggested a number of methods for the chemical utilization of seaweeds[224], but he did not succeed in obtaining pure alginate. Purified alginic acid was prepared by Krefting in 1896[225], and by Hoagland and Lieb[226]. Later investigators determined many of the properties of the salts of this acid. The alginic acid molecule contains mainly uronic acids[227-228] and identification of the uronic acid was studied by various investigators[229-231]. All found D-mannuronic acid in the hydrolysate of alginate. The nature of the glycosidic bond between the residues in the uronic acids of alginate was discussed by several researchers[232-234], Lune and co-workers[235], base on X-ray studies suggested the structure is similar to cellulose and pectic acid, in that it is connected via 1-4 glycosidic bond. The first chemical evidence of the 1-4 linkage in alginate molecules was given by Hirst and co-workers[236-238], by methylation of partially degraded alginate and Astbury's[239] X-ray analysis of alginic acid fibres confirmed the long chain structure.

In 1955 Fischer and Dorfel found a different uronic acid residue as well as mannuronic acid which was later identified as L-guluronic acid[240]. This was confirmed by Drummond and co-workers[241,242] and Whistler and Kirby[243]. The uronic acid residues are linearly linked together via 1-4 linkage as a long chain and no evidence of other forms of linkages or branching was observed[244,245].

The presence of three kinds of polymer segments in alginic acid from various brown algae has been shown by mild acid hydrolysis[246-248] One segment consists essentially of D-mannuronic acid units, a second of essentially L-guluronic acid units and the third segment consists of alternating D-mannuronic acid and L-guluronic acid residues[249] (Figure 8).

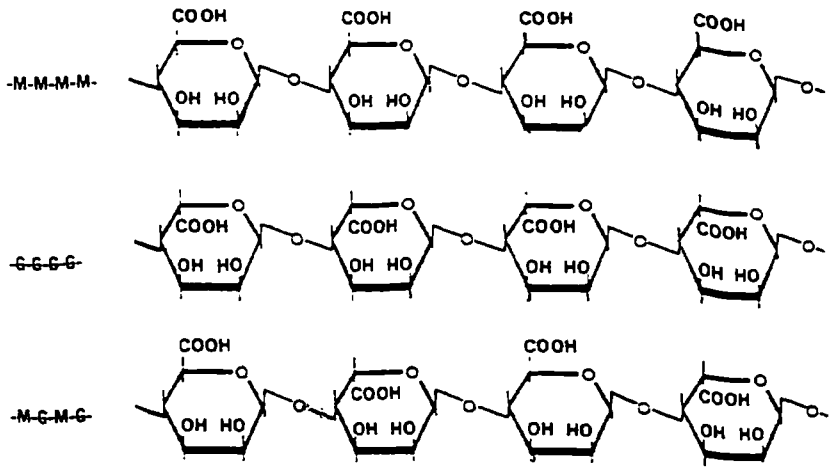


Figure 8: Structure of the polymer segments contained in alginic acid

Further studies using mild acid hydrolysis showed that some parts of the alginate molecule were broken down more readily than others, and furthermore that the part which resisted hydrolysis could be fractionated into two components. One made up almost entirely of

mannuronic and the other of guluronic residues[246,247]. The readily hydrolysed part was made up of roughly equal parts of the two uronides which may be randomly distributed or may make up an alternating sequence. Alginates can therefore be considered as block polymers. The different parts are referred to as M blocks, G blocks and MG blocks. The differences in composition and fine structure account for the differences in properties and functionality of alginates isolated from different species of brown algae. Manufacturers and users have long been aware that changing the type of seaweed used will give quantitative differences in the behaviour of alginates. An explanation for this can now be found because of different proportions of mannuronic and guluronic acid residues, and more particularly that proportion of G blocks[248], although finer differences no doubt depend on the size and arrangement of the blocks. The proportion of the two types of residues and their arrangement into block depends on the species[240], the condition of growth[250], and the anatomical part of the plant from which the alginate is obtained[251]. The composition also depends on the time of harvesting, and to some extent with the age of the plant tissue.

Main Weed Sources

Sodium alginate has been found in all species of brown seaweed but only few which can be obtained at suitable centres in large quantities are used commercially. The most important species are *Macrocystic pyrifera* (Pacific coast of America), *Ascophyllum nodosum* (Europe), *Laminaria digitata* and *Laminaria hyperborea* (Europe and Japan), *Ecklonia* spp. (South Africa) and *Sargassum* spp. (South East Asia). Algal species commonly used for alginate extractions include *Ascophyllum nodosum, macorcystis pyrifera* and various of the *Laminaria.*

A possible future source of alginate is through microbial production, using bacteria such as *Azotobacter vinelandii*[252,253], and *Pseudomonas euruginosa*[254,255]. These organisms produce

alginate which has a proportion of the C-2 and/or C-3 of the M-residues hydroxyl groups which are acetylated. The level of acetylation is variable[256,257]. Though it cannot be ruled out that acetylated G-residues already exist. Therefore O-acetyl groups are the most characteristic feature distinguishing them from the algal polymers.

A. vinclandii alginate was shown to have a block-copolyeric structure similar to that of the algal product[258]. However, in contrast, reports by Sherbrock-Cox, *et al*[255] and Skjak-Break *et al*[257], have shown the complete absence of consecutive L-guluronic acid residues in *P. aeruginosa* alginate. This absence of G-blocks gives an elastic, rather than brittle polymer[255], especially in the presence of increased calcium ion concentration. *A. vinelandii* produces an alginate containing 93% guluronic acid[259].

The yields obtained from bacterial sources however do not suggest that they would provide an economic source of the product.

Alginates from algal were first prepared by Stanford[223], and his methods form the basis of those used commercially at the present time. The main improvements have been in the methods of purification. Bashford *et al*[260] investigated a preparation of calcium alginate using *Laminaria digitata* and *Ascophyllum nodosum* source. They were concerned with the effect of the various treatments on the colour, purity and viscosity of the product. Rose[261] used Canadian brown algae and he investigated the effect of pre-treatment with both $CaCl_2$ and mineral acid. He determined the yield, viscosity and the amount of bleaching agent necessary to obtain a colourless product.

Mannuronate and Guluronate Ratio (M/G)

Alginate with a very high content of mannuronic acid (85%M) can be extracted from fruiting bodies of *Ascophyllum nodosum*, while older tissue of the same algae can contain as much as 40% guluronate (G).

Alginate with a high content of guluronic acid and a high content of homopolymeric GG blocks can be isolated from the stipe of the kelp *Laminaria* hyperborea. This is one of the raw materials used by Kelco International. It contains up to about 65% G blocks, whereas other seaweeds growing around the British Isles have a high proportion of mannuronic acid. By making a suitable selection, a series of alginates with the full range of available properties can therefore be produced from these raw materials. Table IX shows the differences in properties and composition of alginic acids obtained from commercially important brown algae, and Table X summarises the properties of polyuronate acid segments isolated from brown algae.

Determination of M/G ratio is very important, because it is closely related to the gel strength produced. In general, high G alginate produces strong brittle gels, while the high M alginate provide weaker more elastic gels. In table XI the M/G ratio from some common brown weeds are given[262], while in table XII the M/G ratio for different part of *L.hyperborea* tissue is given[262].

The theoretical equivalent weight of alginic acid is 176, but the bond water within the molecule results in measured values to 194[250]. The difference between the actual and theoretical values is most easily accounted for by water which cannot be removed by the normal drying methods. This has been found that sharp X-ray diagrams can only be obtained

Table IX: **Mannuronic acid (M) and Guluronic acid (G) composition of alginic acid obtained from commercial brown algae**

Species	M Content (%)	G Content (%)
Macrocystis pyrifera	61	39
Ascophyllum nodosum	65	35
Laminaria digitata	59	41
Laminaria hyperborea (stipes)	31	69
Ecklonia cava and *Eisenia bicyclis*	62	38

Table X: **Properties of polymannuronic acid, polyguluronic acid and alternating segments in alginic acid from brown algae**

Source	Alternating segment (%)	Polymannuronic acid segment (%)	Polyguluronic acid segment (%)
M. pyrifera	41.7	40.6	17.7
A. nodosum	41.0	38.4	20.7
L. hyperborea	26.8	12.7	60.5

Table XI: M/G ratio from some common brown weeds

Species of weed	M/G ratio
Fucus serratus	1.30
Ascophyllum nodosum	1.85
Laminaria digitata	1.45
Laminaria hyperborea (fronds)	1.35
Laminaria hyperborea (stipes)	0.65

Table XII: M/G ratio from different tissues

Species of weed	M/G ratio
Laminaria digitata	
New fronds	2.30
Old fronds	1.35
Stipes	1.15
Laminaria hyperborea	
New fronds	1.90
Old fronds	1.25
Stipes	0.60
Fucus vesiculosus	
Young tissue	2.20
Old tissue	0.60

when there is some moisture in alginic acid fibres[239]. Atkins *et al*, found one molecule of water to be associated with each acid residue in the polyguluronic acid structure[263].

General Physical and Chemical Properties of Algin Solution

Solubility:

Whether a given alginate is soluble in water depends on the nature of the cation associated with the carboxyl group. Dissolved in water, alginates have properties similar to those of hydrophillic colloids. In distilled water, pure alginates form smooth solutions having long flow properties. The solution properties are dependent on both physical and chemical variables. An alginate solution has a higher viscosity than that of a simple substance of the same concentration. Alginates are polyelectrolytes (polyanions) since they are large molecules and in solution carry negative charge.

It is generally found that alginates are either practically insoluble or completely miscible with water, unlike substances of low molecular weight where the solid will exist in equilibrium with a saturated solution containing some amount of the dissolved solid. This is typical of high polymers, but sometimes the highly polymerised fraction of the alginate may remain undissolved in a solution of the polymers with less degree of polymerisation.

The alginates of the alkali metals, the ammonium cation and low molecular weight organic bases are soluble but alginic acid and its salts of di- or trivalent metals are insoluble with the exception of magnesium salts. Addition of sufficient highly ionised acid or soluble salts of di-or trivalent metals brings about the precipitation of alkali metal alginates.

Alginates are essentially hydrophillic and the simple alginates are insoluble in common non-

aqueous solutions. The addition of water miscible liquids such as ethyl alcohol, to aqueous solutions of alginates cause them to precipitate. For sodium alginate, precipitation occurs by the addition of 20% to 30% of ethyl alcohol. The amount of alcohol also varies due to different bases that may be associated with the alginate. The insolubility of these alginates is a consequence of their polymeric nature and the way in which the carboxyl and hydroxyl groups are arranged.

Stability of Alginates

Like other polysaccharides, alginate is only stable under certain conditions. There are several factors that cause and contribute to the degradation of alginate. If the alginate is very highly polymerised then it can be depolymerised (broken down) to an alginate of lower molecular weight which are stable under normal conditions of temperature.

Complete degradation of alginates to uronic acids, requires heating in the presence of acid. This also causes some breakdown of the uronic acid, the guluronic acid being decomposed at a faster rate than mannuronic acid. Mannuronic acid has, in fact, been isolated from the hydrolysis products[264,265].

Alginate salts and the free acids are stable, sodium alginate of degree of polymerisation (D.P.) ~500 has been stored for three years at 10-20°C with no observable changes. Alginic acid of lower molecular weight with a D.P. ~40 remains virtually unchanged at the above temperature for several years[265]. Therefore the rate of depolymerisation of an alginate solution is greater, the more highly polymerised it is and can even become serious at higher temperature. The rate of depolymerisation is also greater in the presence of moisture than in the dry state. Alginate solutions are fairly stable at pH 6-8.

The presence of acid or alkali which bring the pH <5 or >9 accelerates depolymerisation. Albershem *et al*[266], demonstrated that pectin degraded rapidly either in alkaline or neutral solution and this degradation leads to the formation of unsaturated uronic acid derivatives. Enzymic degradation studies on pectin[267], chondroitin sulphate[268] and alginic acid[269,270], have been shown to give rise to unsaturated compounds and the reaction is supposed to be an elimination reaction. It appears, therefore, that almost all polysaccharides undergo certain depolymerisation reactions.

The presence of auto-oxidisable compounds such as phenol, ascorbic acid, thiol and certain metallic ions such as Fe^{2+} can facilitate depolymerisation of alginate solutions. There are also certain stages where degradation can even occur during the preparation and isolation of alginate.

Molecular Weight

A large number of techniques have been employed for molecular weight determination. In seaweeds, the alginates (as alginic acid) can be very highly polymerised, as shown by the high viscosity solutions produced under controlled extraction techniques.

Using different preparation techniques for the type of weeds and varying the method of extraction, a variety of molecular weight alginates can be obtained. These molecular weights have been measured in a variety of ways, namely viscometry, osmometry[271], ultracentrifugation[272], (sedimentation/diffusion) and light scattering[273-276].

Commercial high viscosity alginates have molecular weights of about 150,000 corresponding to a degree of polymerisation (D.P) of about 750.

Viscosity

A knowledge of the rheological parameters of gums used as stabilizers is important because of their effect on the flow properties of the food products as well as their influence on the mouth feel and textural properties. Because viscosity is basic to the enormous usage of these substances, the most common way of characterising them is by the measurement of their viscosity in an aqueous solution.

Due to the composition of alginate, the viscosity of alginates varies from algae species to species and from sample to sample, depending on the extraction procedure. The physical variables which affect the viscosity and flow characteristics of alginate solution depends on a number of physical and chemical factors such as degree of polymerisation, concentration, temperature, pH, shear rate, presence of other substances in solution and the presence of di- or trivalent metal ions and monovalent salts.

A statement of viscosity must give the temperature at which the measurement was made. Specifications for alginates generally include the viscosity measurement of solutions at either 20°C or 25°C. The nearest standard temperature is taken as 20°C. The viscosity of alginate solutions is important, since the viscosity of extremely dilute solutions gives information about the size and shape of the molecules. Alginate solutions, like other polysaccharides, decrease in viscosity with an increase in temperature. A decrease in temperature causes a viscosity increase in an alginate solution but does not result in gel formation.

The relationship between viscosity and concentration is rather complicated for chain molecules. The viscosity of a colloidal solution with a hard spherical shape does not depend on the size of the particles, but it is determined by the volume fraction of the colloid on the

shape of the colloid particles[277].

The viscosity of linear polymers is markedly dependent upon the volume occupied by the chain molecules. It is known that the reduced viscosity varies with concentration, higher concentrations giving higher values of specific viscosity. The viscosity of linear polymer depends on chain length and chain stiffness[278-280].

The intrinsic viscosity of an alginate is a measure of the volume and shape of the space occupied by the molecules in solution. The molecular weight is only one of the factors involved. As viscosity is largely determined by the length of molecules in solution, those that are composed of stiffer molecules produce a higher viscosity than more flexible ones of the same molecular weight.

Alginates are much less flexible than many polymers. It has been found that the G blocks (guluronic acid residues) are more stiff than the M blocks (mannuronic residues), which are stiffer than the M-G blocks[281]. The viscosity of a dilute sodium alginate solution is depressed by addition of monovalent salts. As is typical with polyelectrolytes, the algin polymer contracts with increasing ionic strength of the solution, the maximum viscosity is attained at a salt concentration of 0.1mol dm^{-3}.

As the concentration of the algin is increased, the electrolytic effect is decreased, except for alginates high in calcium. As the concentration of the salt is increased, the viscosity of the solution may increase. This effect becomes most evident after prolonged storage. Polyvalent cations (with the exception of magnesium) react with the algin polymers and cause cross linking. As the polyvalent ion content is increased, thickening, gelation and finally

precipitation occurs.

Certain polyvalent ions (zinc, aluminium and copper) form complexes with algins in the presence of an excess of ammonium hydroxide. If the ammonia is evaporated from the system, the insoluble metal alginate is formed. Calcium is the polyvalent cation most commonly used to change the viscosity and gel characteristics of algin solutions. Calcium can also be used as a precipitating agent, for the formation of insoluble filaments and films. Sodium alginate forms solutions of high viscosity, even at low concentrations, due to its high molecular weight and the rigid nature of the molecules.

The viscosity is constant between pH 5-10. Although sodium alginate solution appears to tolerate high pH conditions, long-term stability is poor above about pH 10. At higher values of pH, β elimination and hydrolysis result in depolymerization with an accompanying loss of viscosity. Below pH 4.5 the viscosity increases and precipitation occurs, below pH 3.0.

The effect of pH on the viscosity is affected by the presence of other components. Sodium alginates with some residual calcium content, gel at a pH 5.0 whereas sodium alginates with minimal calcium content do not gel until the pH reaches 3 to 4. Estrification reduces the ionic character of the alginate molecule and hence increases its tolerance to low pH. Propylene glycol alginates are compatible and stable at pH 3-4.

Structure of Alginate and Comparison to Other Polysaccharides

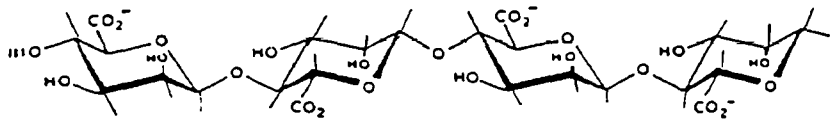
The uronic acid residues in alginate, namely β-D-mannopyranosyl and α-L-gulopyranosyl uronic acids occur as regular 1→4 linked sequences in alginate[282]. It is found that heteropolymeric mixed sequences of both uronate monomers are always present, beside the

homopolymeric blocks[283] (Figure 9).

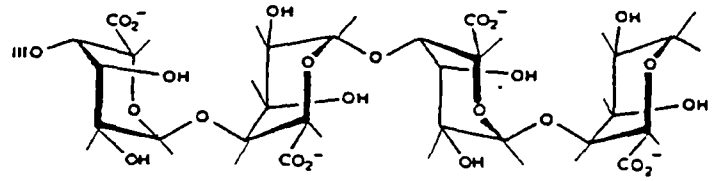
Comparisons of the structure of certain polysaccharides (Figure 10), suggests that poly β-D-mannuronate will show some properties similar to cellulose. X-ray examination shows that alginic acid, like cellulose, is largely in the crystalline state and has a very regular structure and so there is great opportunity for hydrogen bonding to occur between the polymer chains at regular intervals. This would mean that more energy would be needed to separate the molecular chains. Pectin on the other hand is the partial methyl ester of pectic acid, occurs naturally as α-D galactopyranosyl units. In this case, the energy required to separate the molecular chains is much less than for alginic acid and cellulose. Removal of the methyl group, by hydrolysis, gives pectic acid which is insoluble in water and has similar properties to alginic acid. In the same way some derivatives of cellulose and of alginic acid (where there are irregularities in the chain) are soluble in water. For example the partial methyl ether of cellulose and the partial propylene glycol ester of alginic acid. Alginic acid reacts relatively rapidly under mild conditions with alkylene oxides to give water soluble esters e.g. propyle oxide.

The presence of the acid groups in alginic acid affords a very easy method of bringing it into solution. The pH required to precipitate alginic acid from a solution of sodium alginate depends on the degree of polymerisation, the proportion of mannuronic and guluronic acid residues and their arrangement in the molecule. Undegraded alginates are precipitated over the pH range 3.5-2.5, alginates which have a high concentration of guluronic acid or a high degree of polymerisation are more readily precipitated. Studies on calcium induced gelation of pectin at different degrees of substitution give strong evidence that galacturonate sequences are important for Ca^{2+} binding and not methyl galacturonate[283]. A decrease in the number

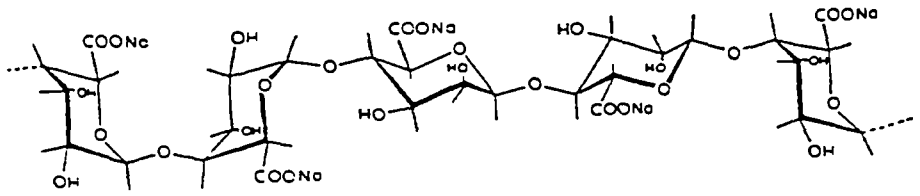
Figure 9 **Primary structure of alginate (M = D-mannuronic acid, G = L-guluronic acid)**



1–4 ß D – Mannuronic

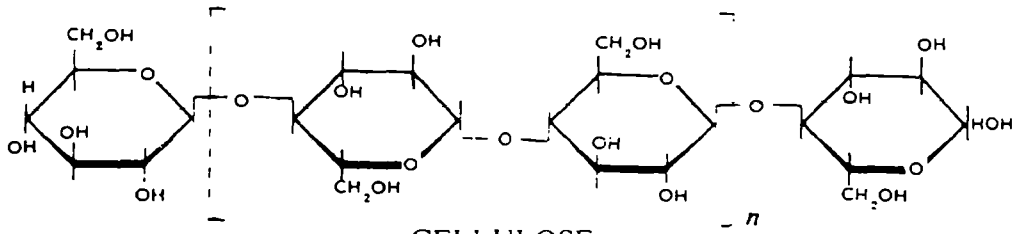


1–4 α L – Guluronic

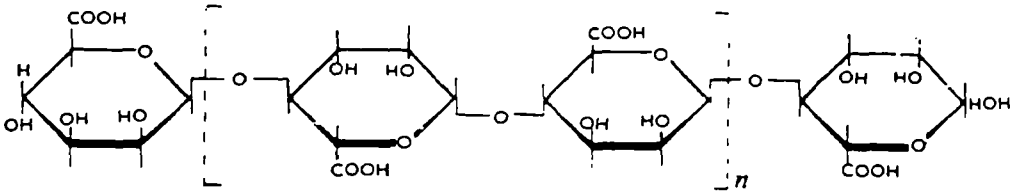


G–M

Figure 10 Comparison of the structure of certain polysaccarides



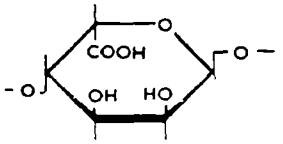
CELLULOSE

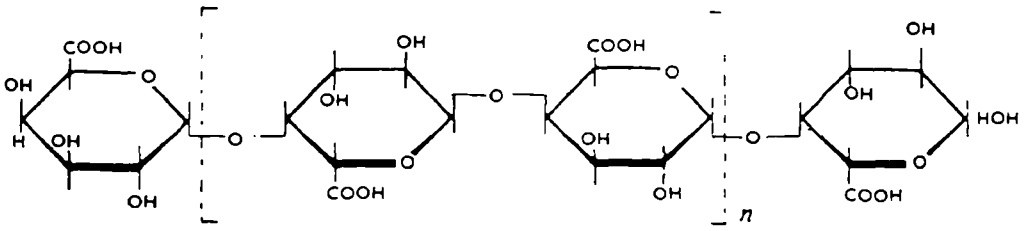


ALGINIC ACID (with D-Mannuronic acid units)

or with some

L-Guluronic acid units:—





PECTIC ACID

of methyl groups esterified causes an increase in gel strength, due to higher intermolecular association.

Because of the similarity in the geometry of the ion binding sites in alginate and pectin, pectin gelation can be interpreted in the same way as for polyguluronate segments in alginate.

Rheology of Algin Solution

Concepts of Rheology

As described in the previous chapter, rheology is defined as the branch of physics concerned with the deformation and flow of matter. Some of the earliest observations on the rheology of water are attributed to Sir Isaac Newton. The fluid motion described by Newton is known as steady shearing flow in which infinitely thin parallel planes of fluid slide over each other in response to a constant force applied in the direction of the sliding planes. Shear stress is defined as the force per unit area of the fluid planes and shear rate as the velocity difference per unit thickness of the fluid. The proportionality constant between the shear stress and the shear rate arising from the "lack of slipperiness" within the fluid, is called viscosity. The units of shear stress may be dynes/cm^2 and shear rate is in sec^{-1}. Hence, viscosity is expressed as dyne sec/cm^2, which is defined as the poise. Commonly, viscosities of fluids are stated in centipoises (1cps = 0.01 poises) to provide a convenient comparison with the viscosity of water, which is approximately one centipoise at room temperature.

The flow properties of water and similar fluids that exhibit a constant ratio between shear stress and shear rate are said to be Newtonian. A plot of the shear stress/shear rate relationship, known as rheograms, would be a straight line. For non-Newtonian fluids, the ratio of shear stress/shear rate at any point is known as the apparent viscosity.

The most common type of non-Newtonian behaviour is pseudoplastic flow in which the fluid exhibits shear thinning over a wide range of shear rates. As earlier described (Ch. 1) there are different types of flow behaviour for non-Newtonian flow known as dilatant (shear thickening), thioxotropy and rheopectic (time dependent).

Flow behaviour of solutions is also influenced considerably by temperature and solute concentration. Viscosity decreases with an increase in temperature. Dilute solutions and solutions of low molecular weight compounds usually have Newtonian flow properties, while non-Newtonian flow is common for high molecular weight polymeric solutions, even at low concentrations. With increasing concentration, apparent viscosity increases and the shape of the rheogram often changes, depending on the nature of the solute and the presence of other components in the solution.

Viscosity Measurement

Many types of viscometers (or rheometers) have been developed over the years. Several viscometer designs are suitable for measuring viscosities of Newtonian fluids. However, only a few are capable of characterising non-Newtonian fluids, which require determination of shear stress and shear rate in steady shearing flow over a wide range of shear rates.

Viscometric designs that may be used for both non-Newtonian and Newtonian fluids are the capillary tube or extrusion rheometer and rotational rheometers with either narrow-gap coaxial cylinder, infinite-gap cylindrical, or cone/plate fixtures.

The coaxial cylinder or couette rheometer shears a fluid in a narrow annular space between a cylindrical spindle and cup, one of which is stationary while the other rotates. Coaxial

cylinder geometry is available in several accessories for the Brookfield viscometer, eg. the Small Sample Adaptor, the UL Adaptor and the Thermosel System.

In addition to providing scientifically defined rheological data (including shear stress and shear rate values), each of these accessories has unique advantages for specific situations. The Small Sample Adaptor, consists of a cylindrical sample chamber and spindle, providing a viscosity range (centipoise) of 5-1,600,000, (Table XIII). It provides a defined system for accurate viscosity measurements of small sample volumes in the order of 2 to 16cm^3. The design of the small sample adaptor allows the sample chamber to be easily changed and cleaned without disturbing the set-up of the viscometer or temperature bath. This means that successive measurements can be made under identical conditions. The sample chamber fits into a flow jacket so that precise temperature control can be achieved when a temperature bath is used. The working temperature range is from -10°C to 100°C. Brookfield disc-type, spindles are frequently used, particularly for quality control tests of industria fluids. Disc spindles are versatile general-purpose spindles for use in containers of 600cm^3 capacity or larger. Disc spindles produce accurate, reproducible apparent viscosity determinations in a wide variety of liquids.

For Newtonian fluids a suitable spindle size and rotational speed combination must be chosen to obtain a reading, which is multiplied by a factor (Table XIV) to give the fluid viscosity. It should be noted that flow curves for non-Newtonian fluids cannot be obtained using disc-type spindles, because shear rate varies across the upper and lower surfaces of the disk from the centre to the outside edge. Nevertheless, disc-type spindle readings can be a useful indication of the consistency of a non-Newtonian fluid if the viscometer, spindle number and rotational speed are specified. Cylindrical spindles (LV #1 and #4, RV/HA/HB #7) provide

a scientifically defined spindle geometry for calculating shear stress and shear rate values as well as viscosity, just like those of disc spindles. Cylindrical spindles are particularly valuable when measuring non-Newtonian fluids and are applicable to any Brookfield viscometer model with the use of the appropriate range.

Viscous Behaviour of Algin Solutions

Sodium alginate forms solutions of unusually high apparent viscosity even at low concentrations because of its high molecular weight and the rigid nature of the molecules. The solutions are pseudoplastic and show shear thinning over a wide range of shear rates, particularly at high solution concentration.

The Brookfield Viscometers

Brookfield laboratory viscometers are available in two basic types: dial-reading and digital. Mechanically, there is no difference between dial and digital models, the only variation is the manner in which the viscosity reading is displayed. The dial-reading type is read by noting the position of a pointer in relation to a rotating dial, the digital type is read by means of a 3-digit LED display.

The Brookfield Dial model gives a mechanical reading of % torque. The torque of the spring is known and as the friction of the fluid is measured using a changeable spindle, the resistance on the spindle winds up the spring. The viscosity of the fluid is then recorded by multiplying the dial reading by the known factor for the spindle and speed combination used (see table XIV). This is the very basic and most inexpensive model, operation is slow and fiddly! This model is not recommended for new users.

Table XIII: Range data, applicable to dial and digital viscometer for small sample adaptor

Spindle and Chamber	Viscosity Range (CPS) LVT	Shear rate (sec^{-1}) N=RPM	Sample Volume (Ml)
SC4-18/13R	5-10,000	1.32N	8.0
SC4-31/13R	50-100,000	0.34N	10.0
SC4-34/13R	100-200,000	0.28N	10.0
SC4-16/8R	200-400,000	0.29N	4.2
SC4-25/13R	800-1,600,000	0.22N	16.0

Table XIV: Spindle series of L.V. viscometer with corresponding rotational speed and factors

Spindle speed	1	2	3	4
	Factor			
0.3	200	1M*	4M	20M
0.6	100	500	2M	10M
1.5	40	200	800	4M
3.0	20	100	400	2M
6.0	10	50	200	1M
12	5	25	100	500
30	2	10	40	200
60	1	5	20	100

*M = 1000

The digital viscometer DV11 models give a direct viscosity reading (centipoises), shear stress (dynes/cm^2) as well as % torque/scale readings (Brookfield). All digital models are continuously sensing and so give rapid results. In addition the digital viscometer includes a 0-10mv output that can be connected to a variety of devices, such as remote displays, controllers, chart recorder, printer on computer. Connection to a computer ensures rapid data collection and processing of results. Flow curves can be produced and data stored.

Brookfield Digital viscometers are available in all LV, RV, HA and HB, 8-speed models. They are compatible with all Brookfield accessories and can be applied (on a model to model basis) to all existing Brookfield viscosity specification. Speed changes are affected by a transmission having eight speeds. The round speed control knob rotates both clockwise and counter-clockwise. Maximum speed (rpm) will be set at full clockwise rotation and minimum speed at full counter-clockwise rotation. The speed setting is indicated by the number on the knob located opposite the button on the viscometer housing (see Table XIV). Although not absolutely necessary, it is advisable to change speeds while the motor is running.

Table XV shows a range of data for dial and digital models including the number of speeds, number of spindles and spindle entry, (SPDL, which is the spindle number entry access key). It is also necessary to enter the speed [rpm], number of ranges, minimum and maximum viscosity and the spindle number (the number of the spindle to be used). The spindles are attached to the viscometer by screwing them to the lower shaft. The lower shaft should be held in one hand and the spindle screwed to the left (left hand thread). Spindles can be identified by the number on the side of the spindle nut. The digital display on this viscometer reads from 00.0 - 99.9 in the % mode. Overrange is indicated by "EEE",

underrange is "---". Floating point display is used for the viscosity (CPS) and shear stress (ss) modes. These can be changed at any time without affecting the viscosity measurement.

Low Reading Indicator

If the viscometer reading is less than 10% of the full scale range the low LED indicator comes on. The purpose of this indicator is to indicate to the operator that the measurement is on the low end of the full scale range. This is especially important when using the CPS and SS modes. The viscometer calculates viscosity and shear stress at any upscale reading above zero, and it is recommended to take readings above 10%.

LV Model (LVTDV-II)

This instrument is calibrated to Bureau of Standards values on the basis of immersion in an infinite body with the guard leg attached. It is accurate to within 10% of full scale when the spindle is centred in any container over 2-3/4" in diameter. Using the viscometer in smaller containers reduces the effective range of measurement provided by the #1 and #2 spindles. The calibration of the #3 and #4 spindles is unaffected by the size of the container used as long as the guard leg is attached. The various models of the Brookfield viscometer are recommended for high, medium and low viscosity application. Multiple speeds and interchangeable spindles on each viscometer provide many viscosity ranges for flexibility in application.

Selecting the right model will ensure maximum sensitivity and accuracy in the range of viscosities measurement: eg. the LV spindle, set for low viscosity up to 2,000,000cps can be used for different types of materials, such as adhesives, chemicals and soups. The RV spindle set for medium viscosity can be used for creams, food products, gums, etc. and the

HA/HB spindle, set for high viscosity is used for asphalt, chocolate, gels, etc.

The small sample adaptor with a cylindrical spindle enables the viscosity of small volumes (2-16cm^3) to be accurately measured (table XVI, also see table XIII). It is necessary that the following information is always recorded when making viscosity measurements: viscometer model, spindle, rotational speed, container size or dimensions, sample temperature, sample preparation procedure and whether or not the spindle guard was used. Spindle guards are provided only on LV and RV models of the dial-reading and digital viscometers with standard spindles.

When a test must be performed at several speeds, it is necessary to select a spindle that produces on-scale readings at all of the required speeds. This may necessitate using a dial or display reading less than 10, which is acceptable as long as the reduced accuracy of such a reading is recognised. As mentioned earlier, readings should be between 10 and 100, the accuracy improving as the reading approaches 100. If the reading is over 100, a slower speed should be selected and/or a smaller spindle. Conversely, if the reading is under 10, a higher speed and/or a larger spindle is selected. The spindle should be immersed up to the middle of the indentation on the shaft. A sample container size with an inside diameter of 3¼ inches (83mm) or larger is recommended for use with standard viscometer models and/or a 600ml beaker. The use of a small container results in an increase in viscosity readings, particularly with the #1 and #2 spindle. When using a smaller container, the simplest approach is to report the dimensions of the container and ignore the probable effect on the calibration, provided the same size container is used for all subsequent tests.

The sample fluid should be free from entrapped air and be at constant and uniform

Table XV: Data range for dial and digital, for speed, spindle, spindle entry and min, max viscosity

Dial model Digital	LVT: LVTDV-I LVTDV-II
No of speeds	8*
No of spindles	4 (1, 2, 3, 4)
No of ranges	32
Min viscosity (centipoise)	15
Max viscosity (centipoise)	$2MM^{x}$
SPDL	61,62,63,64

* As shown in table XIV

^{x} = M = 1000

Table XVI: Small sample adaptor for digital LV series with SPDL entry number

Spindle	SPDL Entry
SC4-18	18
SC4-31	31
SC4-34	34
SC4-16	16
SC4-25	25

temperature. Homogeneity of the sample is also important. Finally the viscometer should be run until a constant reading is obtained. A number of procedures can be employed to obtain a satisfactory reading. In some cases up to 5 minutes must be allowed for the reading to reach apparent equilibrium or until the reading remains relatively constant.

Practical Application of Alginates

Alginates are widely used and are accepted additives for the food industry. Alginates are non-toxic (the toxicological properties of alginates have been extensively investigated and summarized)[285]. It has been shown in man[286,287] and in animals (cow's milk)[288,289] that addition of sodium alginate to the diet reduces the absorption of strontium without materially affecting the absorption of calcium[290]. This could be of value in reducing the injury caused by accidental ingestion of radioactive strontium.

Further studies with different alginates have shown[291,292], that the reduction of strontium uptake is greater the higher the proportion of guluronic residues in the alginate, while the degree of polymerisation is of little importance.

Sodium alginates have received much attention as thickening, gelling, emulsifying and stabilising agents, as well as a dietary food. Historically, alginate entered the field of food application as an ice-cream stabiliser and it is still widely used. It is also used as stabilisers in processed cheese, whipped cream, as coatings for frozen fish or chicken, as synthetic sausage skins, icing and topping for cakes. Synthetic potato chips have also been patented from a blend of a wet dispersion of sodium alginate, starch, potato flour and calcium lactate. Synthetic fruits (cherries) are made by allowing drops of mixture of suitably coloured and flavoured sodium alginate solution to fall into a bath containing a solution of calcium

chloride.

The application of alginate are not limited to the food industries. For instance, sodium and ammonium alginate are widely used as emulsifying agents or emulsion stabilisers in paints and dyes. Alginates have been found to improve the flow quality and appearance of paints, making the latter more durable and resistant to weathering. Almost half of the total world alginate product is consumed in the textile industry where it is used as a thickening agent for print pastes.

Alginates are used for numerous technical application, such as a coagulant for sludge formation in the internal treatment of boiler water and as co-flocculant in water purification, especially drinking water. In addition, alginate has also been used as an emulsifier on photographic materials, and as a tackifier in insecticide and pesticide aerosols. Alginates have also found usage in fabric and pharmaceutical industries, in the production of explosive materials, as well as in the purification of crude oil and tar, and thickening water for fire fighting.

Alginates, like other natural organic substances are attacked by various microorganisms. Highly purified alginates do not support the growth of organisms but most solutions used in practice contain sufficient nitrogen and salts to allow growth to take place. Alginate solutions are conveniently protected from microbiological attack by the use of preservatives. Formaldehyde is particularly effective, and chlorinated phenols, esters of p-hydroxybenzoic acid, phenyl mercuric acetate and other bactericides can be used. Heat treatment can also be used when applicable, but some degradation of the alginate must be expected.

The FAO/WHO Joint Expert Committee on Food Additives (JECFA) has established an acceptable Daily Intake (ADI) of 0-50mgs/kg for alginic acid and its salts[293], and 0-25mgs/kg for propylene glycol alginate[294].

Gel Formation Using Sodium Alginates

From the commercial point of view, the most important properties of alginates are their ability to form viscous solutions and gels. Gels can be formed from alginic acid using controlled amounts of most divalent and trivalent cations. The final use of the gel governs the method of its preparation and final composition. Addition of a soluble calcium salt to a solution of alginates which leads to gel formation is the most common of these gels. The gelling characteristic is, as with other properties of algin solutions, strongly influenced by the ratio of guluronic to mannuronic acid (the results of the M/G ratio have been published for alginate from many different species)[295,296]. Alginates with low M/G ratios produce rigid and brittle gels that are subject to synersis. The different gelling properties are due to the two poly-uronic acids having different conformations in the system. The amount of Na^+ and Ca^{2+} ions as well as temperature and degree of polymerization are also very important factors influencing the final gel characteristic.

The primary model of interchain association in alginate gels *in vitro* is by dimerisation of polyguluronate sequences with interchain chelation of calcium or related divalent cations[297]. Under conditions of free availability of Ca^{2+}, further association of these dimers and perhaps to some extent of mixed chain sequences may occur[298]. There is no evidence of any cation induced association of polymannuronate. Knowledge of the chemical structure of alginic acid has made it possible to control chemically, by addition of Ca^{2+}, the gelation of alginates in foods. The calcium for gelation can be selected from salts such as phosphate, nitrate or

tartrate. The pH value can also be controlled. These characteristics make alginates very suitable for controlled gel formation with aqueous systems. The alginate gel is formed by steady and uniform release of calcium or other cations and the result is a homogenous mass of insoluble and greatly hydrated alginate gel. It is reasonably stable towards the normal changes of the average environment, and this is often a valuable practical advantage with many applications.

Gel formation is possible with most soluble alginates and many cations. In many instances jelly formation is brought about using a combination of acid and calcium salts according to the reaction mechanism and method used. Alginate gels can usually be made using a number of components. Moreover, the way in which the ingredients are mixed with one another may be decided by the processing conditions as well as variation of the amount of ingredients. Most alginate gel compositions are usually prepared and used at room temperature. Sometimes, however, it is necessary to use a hot solution containing the alginate and gelling agent ingredients in such proportions that gelling does not take place until the mixture cools. For example, for some jellies the most satisfactory results are obtained by using manucol types of sodium alginate rather than the higher gel strength manugel types. Therefore the nature of the gel formed will depend on all of these factors. However most types of alginate can be made into aqueous gels, given the right conditions, but as mentioned above, the alginate composition affects the nature of the gel formed, bearing in mind that changes in the M/G ratio influences the physical and hydrodynamic properties of alginate[275,276]. In general, alginates with a high proportion of G blocks tend to give firm but rather brittle gels, while those with low G blocks give weaker but more flexible gels, possibly because the guluronate rich fraction is characterised by a more rigid extended conformation compared to mannuronate[275].

The stiffness associated with the L-guluronate rich fraction is attributed to either stronger carboxylate-carboxylate repulsion between adjacent α-linked guluronic acid residues or the decreased flexibility of its glycosidic linkage due to more extensive steric restrictions than is the case of β-linked mannuronic acid residues. Figure 9 shows that in poly α-L-guluronate the axial-axial configuration of the glycosidic linkage leads to a distinctly buckled ribbon structure with very limited flexibility (fibre axis spacing is 8.7A for G blocks and 10.3A for the M blocks)[263]. This type of packing gives evidence of large interstices existing in the molecule, and cooperative interactions between such buckled ribbons will only be strong by filling the interstices effectively with cations or water molecules.

Studies of the solution properties of sodium alginates using MG, MM and GG blocks show that the relative extension of three types of sequence increases as follows: GG-blocks > MM-blocks > MG blocks. Alginates recommended for gel formation are those with the name Manugel, and these have been standardised for gel strength. The manucol grades give lower gel strength but are more suitable for preparing gels by cooling. The amount of calcium required for gel formation is less in acid than in neutral conditions. The nature of the calcium salt and acid used, as well as the type of alginate affects the proportion of calcium in the mixture which combines with the alginate and thus contributes to gel strength.

Syneresis (drainage of free water from the gelled mass) sometimes occurs, particularly if the alginate has been converted very largely into the free acid or calcium alginate form, ie. if the ratio of calcium and hydrogen ions to sodium alginate is high. Calcium alginate gels are now used extensively for the manufacture of a wide variety of commercial products including fabrics, filaments, films, plasticisers and, of course, emulsifying agents.

Their potential usefulness in surgery and wound management was first reported in 1947 by Blaine[214]. He showed that alginates are highly absorbent, gel forming materials, with haemostatic properties. Alginate wound dressings have been used in a variety of forms for many years. It has long been known that more wound healing occurs when a gel is formed at the wound surface and dehydration is prevented[299]. In contact with body fluids, alginates are known to break down to simple monosaccharide type residues and be totally absorbed without any side effects[300]. The wound exudate is converted from calcium salt, which is insoluble, to the sodium salt, which is soluble, facilitating the removal of the dressing by dissolution. Groves and Lawrence (1986)[301] have shown that significant haemostasis can be obtained when calcium alginate is applied to graft donor sites in the immediate post-surgery phase. This has formed the basis for the dressing "sorbsan" which is composed of calcium alginate prepared as a textile fibre. Furthermore the value of calcium alginate gels as a wound interface layer for composite absorbent dressings were investigated. Where it could act to prevent gross dehydration of the wound surface and facilitate dressing removal by preventing dressing adhesion[300]. Alginate in its calcium or sodium salt form also has most widespread uses in the field of oral, nasal and neurosurgery where it is applied in the form of a pack to control bleeding within a cavity.

As alginates, like other natural organic substances, are attacked by microorganisms, the need arose for sterile solutions or gels with properties as similar as possible to those of an unsterilised control. The various doses of γ-irradiation up to the standard sterilization dose (25-30kGy) have been used to assess the effect of irradiation on alginate gels and solutions. The viscosity of sodium alginate solutions were measured before and after irradiation. Water uptake and loss, saline uptake and bending ability (gel strength) were also measured as indicators of dressing suitability.

Hartmann *et al*,[302] have reported that irradiation is not appropriate for sterilization of the contaminated microorganisms in sodium alginate because of decrease in viscosity. This is due to decrease in molecular weight and therefore chain length initiated by the major oxidising radical produced during water radiolysis, the hydroxyl radical $\dot{O}H$.

The extent of depolymerisation can be decreased by adding a $\dot{O}H$ radical scavenger, eg. mannitol, though at the very high doses used for the purpose of sterilization, substantial depolymerisation may still occur.

Xanthan Gum a Bacterial Polysaccharide:

Many useful microbes are directly obtained from the soil or other natural sources and many that produce extracellular polysaccharides are widely distributed in both marine and land environments. The polysaccharides they produce are often complex and of varying composition and conformation. One such microorganism is *Xanthomonas campestris*, a naturally occurring bacterium originally isolated from the rutabaga plant.

The production of polysaccharides, by fermentation, has been used for many years. Xanthan gum, a high molecular weight polysaccharide is produced by fermentation on a glucose medium of the microorganism *Xanthomonas campestris* and was developed in the Northern Research Laboratories of the United States Department of Agriculture (USDA) laboratory in Perria in the early 1960's. This polysaccharide was denoted polysaccharide NRRL B-1459[303] and subsequently was given the generic name "xanthan gum".

Non-food grade xanthan gum was introduced in 1961 by Jeanes and coworkers[303] and substantial commercial production by Kelco began in 1964 under the trade name KELZAN. After extensive animal feeding trials, it was first approved for food use by the Food and Drug Administration (FAD) in 1969[306]. Production of food-grade xanthan gum designated under the name KELTROL began in 1969[307].

Structure and Conformation of Xanthan Gum:

Xanthan gum is structurally very complex. Figure 11 shows its primary structure as proposed by Jansson *et al* (1975)[308]. It consists of the repeating units containing two glucose units, two mannose units and one glucuronic acid unit. The main chain is built up of β-D-glucose units linked through the 1- and 4- positions. The side chain, which contains two

mannose units and one glucuronic acid unit between two mannose units and is linked to every other glucose unit in the three position. It is found as a mixed potassium, sodium and calcium salt.

The terminal β -D-mannose unit is glycosidically linked to the 4-position of β-D-glucuronic acid, which in turn is glycosidically linked to the 2-position of α-D-mannose. The three-sugar side chain is linked to the 3-position of every other glucose residue in the main chain. On approximately one half of the terminal D-mannose residues, a pyruvic acid moiety is joined by a ketal linkage to the 4- and 6-position[308-310]. This has been reported to probably play a key role in the molecular properties of xanthan gum in solution[311]. Finally the non-terminal D-mannose unit carries an acetyl group at the 6-position.

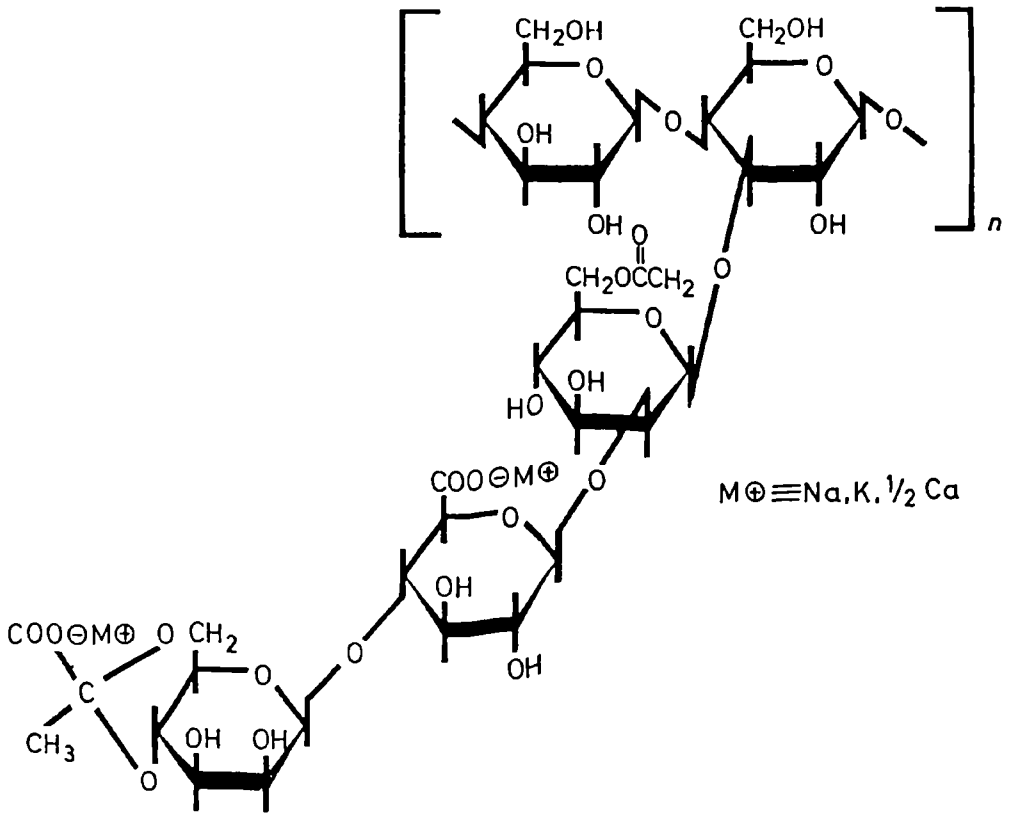


Figure 11: Structure of Xanthan Gum

The amount of pyruvic and acetic acid varies[303,312]. Although the primary structure of xanthan gum is well known the molecular weight and the secondary and tertiary structure of xanthan gum have been and are still a matter of investigation[313-316]. However molecular weight measurements vary from two million daltons[317-320] to as high as 8 to about 50 million daltons.

Water solutions of xanthan gum are extremely pseudoplastic, when shear stress is applied the viscosity is reduced in proportion to the amount of shear (shear thinning)[322,323]. On release of shear the total viscosity recovers almost instantaneously. This behaviour of xanthan gum solutions can be explained on the basis of the helix structure suggested by Rees, 1972, 1973[324,325].

Several workers concluded that xanthan takes on an ordered[315,326-329] single strand and whereas others suggested that it has a double strand[314,320,321,330,331]. However it seems that a xanthan sample may effectively be a single or a double stranded depending on the fermentation process or, more probably, on how it has been treated after the fermentation step. Sato *et al*,[330,331], demonstrated that a sample manufactured by Kelco was a double helix in aqueous solution containing 0.1mol dm^{-3} NaCl. Therefore, the structure and conformation also appears to have key roles in determining many of the unique and useful properties of xanthan gum.

Chemical and Physical Properties of Xanthan Gum Solutions

The structural and rheological properties of xanthan gum dispersions have been investigated widely[311,323,332-337]. Xanthan gum solutions have been described as the most pseudoplastic gum solutions available[332].

Xanthan properties in solution have been studied by many researchers who have characterized its flow behaviour at intermediate shear rates[311,323,332,333,337]. the polymer is an efficient thickening agent whose aqueous solutions are shear thinning[322,323]. The gum is completely soluble in hot or cold water. Of special practical significance is its unusually high viscosity at low concentrations, a factor that contributes to the effective stabilizing properties of xanthan gum. The property of pseudoplasticity (shear rate thinning) is a desirable property in many fluid foods as it results in good suspending properties at low shear rates without rendering the food too viscous to mix or pour at higher rates of shear. Apart from the rate of shear, other factors including gum concentration, temperature, pH, salt concentration and gum source can influence xanthan gum solution properties.

Effect of Temperature on Viscosity:

Xanthan gum solutions show excellent stability at high temperature and are remarkably resistant to thermal degradation. Temperatures as high as 80°C for extended periods has little effect on the viscosity of the gum. This property has practical uses in preparation of hot foods, such as gravies and sauces. The viscosity of these foods may be kept constant while they are heated in a water bath for several hours. Also, the viscosity of xanthan gum solutions is essentially unaltered by temperatures varying from -4°C (25F) to near boiling 79°C (200F). However at temperatures beyond boiling point (120°C) solution viscosity drops by 98%, but it can recover to about 80% of the original viscosity upon cooling. Therefore flow characteristics will be the same in all climate zones.

Effect of pH on Viscosity:

The viscosity of aqueous solutions of xanthan gum is essential independent of pH between 6 and 9, and shows only small changes in viscosity over the pH range 1-13.

Effect of Salts:

Xanthan gum has excellent stability and compatibility with many salts. However high pH influences this compatibility between xanthan gum and polyvalent metal ions, which can often be inhibited or controlled by addition of high levels of monovalent salts and sequestrants such as polyphosphates. The effect of salt on food grade xanthan gum (KELTROL) solutions depends on the concentration of the gum. At low concentration (below 0.15%) monovalent salts (e.g. sodium chloride) cause a slight decrease in viscosity, whereas at higher gum concentrations viscosity increases.

Effect of Enzymes:

Commercially available enzymes such as proteases, cellulase, hemicellulase and amylase do not degrade xanthan gum in the dissolved state. Also xanthan degradation by microbes is rare[338]. But like other polysaccharide gums, xanthan gum solutions will support microbial growth and a preservative such as formaldehyde and methyl p-hydroxy-benzoate is recommended if xanthan gum solutions are stored longer than 24 hours. However in enhanced oil recovery field tests, using xanthan gum as the viscosity controlling agent, xanthan was found to be degraded by microbial (enzymic) activity[339]. Therefore the biodegradation of xanthan gum and the means for its prevention have become important research issues. Cadmus *et al*[340] and Sutherland[341], reported a salt-tolerant bacillus that was able to degrade xanthan gum.

Effect of Acids and Bases:

Xanthan gum dissolves directly in many acids such as 5% acetic acid and remains stable for several months unless the temperature is elevated. This causes acid hydrolysis of the polysaccharide and lower viscosities may result. Xanthan gum is also soluble directly in 5%

sodium hydroxide solutions. Concentrations of sodium hydroxide higher than 12.0% can cause gelation or precipitation. Gelation also may occur in concentrated (above 5%) basic salts such as sodium carbonate, phosphate or metasilicate after prolonged storage. These highly alkaline, thickened solutions have an exceptional viscosity stability. But the best result can be obtained when both acids and alkaline are added to xanthan gum predissolved in water.

Compatibility with Other Gums:

Xanthan gum is compatible with most common gums, but there exists an intense and useful interaction with galactomannans[342], such as locust bean gum, as reported by Schuppner (1971)[343] and guar gum, reported by Rocks (1971)[344]. These react synergistically with xanthan gum to provide increased viscosity or gel formation which can be used for the treatment of wounds.

Guar gum and locust bean gum are polysaccharides composed solely of mannose and galactose. The backbone of both polymers is made up of a linear chain of β-(1-4)-linked D-mannose units. Attached to the backbone, via α-(1-6) links are single unit D-galactose, side chains (Figures 12 and 13)[345]. For guar gum, the mannose-to-galactose ratio is 1.8 and for locust bean gum the ratio is 4. The molecular weight of locust bean gum was reported to be 310,000[347].

The xanthan gum-locust bean gum combination shows a large viscosity increase even at low concentration (between 0.005 and 0.1 percent). At higher concentrations (above 0.5 percent) a cohesive, viscoelastic gel is produced. The maximum gel strength is achieved at approximately equal ratios of the two polymers. The solution pH also influences gel

Figure 12: Structure of guar gum

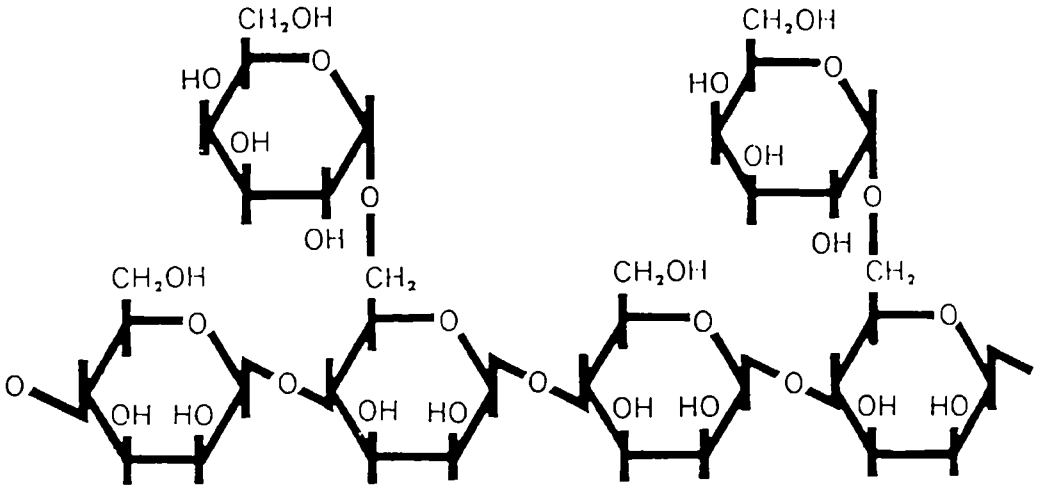
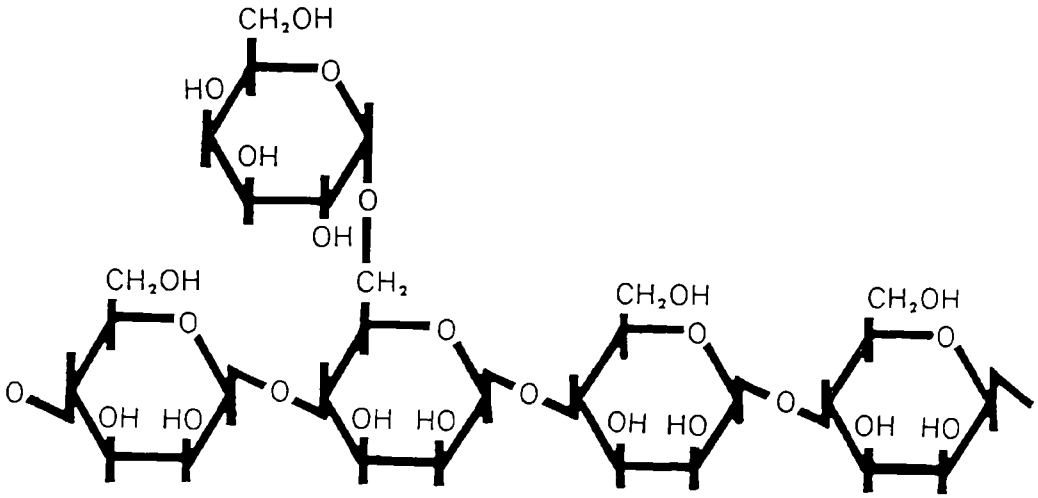


Figure 13: Structure of locust bean gum

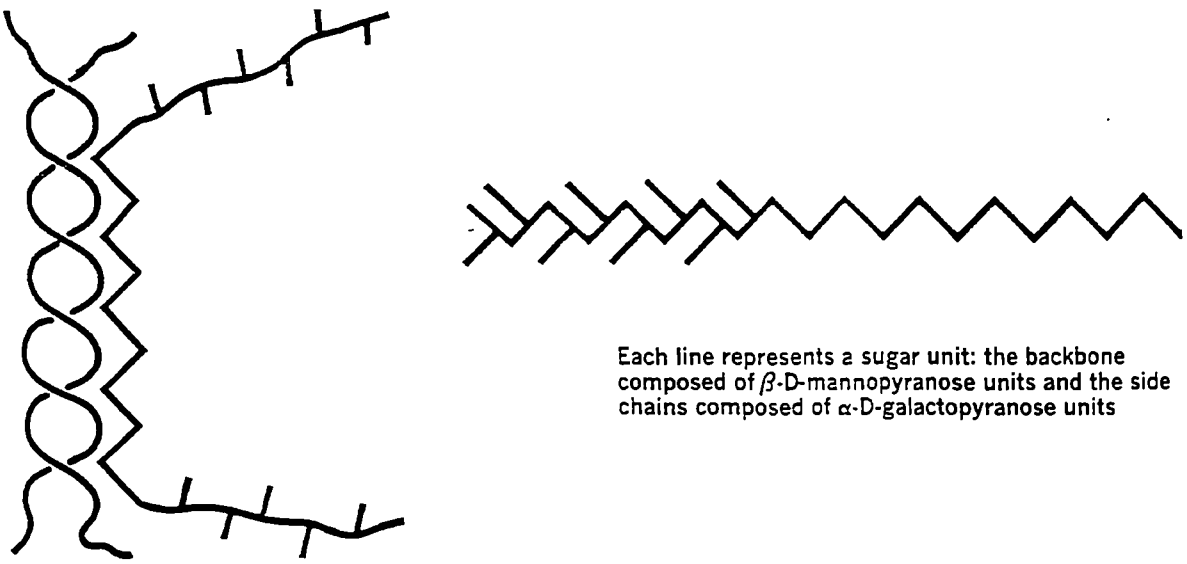


firmness, higher gel strengths are observed under neutral or slightly alkaline conditions, irrespective of the ratio of the two polymers.

On the basis of spectroscopic and rheological data, a model similar to that shown in Figure 14 has been proposed[315,347]. Since galactomannans that are most reactive with xanthan gum have higher proportions of mannose units it is assumed that the smooth, unbranched segments of the galactomannan are involved in the interaction.

As mentioned earlier it has been suggested that xanthan gum contains helical regions and a xanthan gum/locust bean gum gel arises from the interaction between these helices and the smooth, unbranched regions of the locust bean gum to form a cross-linked, three-dimensional network, (Figure 14). Blends of one or both of the above galactomonnans with xanthan gum provide a range of thickening and gelling properties that have found practical significance in food systems.

Figure 14: Possible model for the interaction between xanthan gum and locust bean galactomannan, resulting in gel formation.



Summarizing, xanthan gum is characterized as:

1) uniform viscosity over the temperature range 0-100°C;
2) almost uniform viscosity over the pH range 1.5-13;
3) high viscosities at low concentrations;
4) a high degree of pseudoplasticity over a broad shear rate and concentration range;
5) excellent thermal stability;
6) solubility and excellent stability under both acidic and alkaline conditions;
7) resistance to common enzymatic degradation;
8) compatibility and stability with most common salts;
9) compatibility with other gums and show synergism with guar and locust bean gum for use in a wide range of thickening and gelling applications.
10) soluble in both hot and cold water;
11) form solutions that are easily pumped or poured'
12) contribute excellent mouthfeel to various foods and beverages because of their unique rheological properties;
13) extremely effective emulsion stabilizers;
14) excellent suspension for insoluble solids oil droplets;
15) impart freeze/thaw stability.

Safety Properties and Regulatory Status:

Xanthan gum is one of the most extensively investigated[348] polysaccharides in terms of toxicological testing and other safety studies. Initial, short-term feeding studies were conducted at the Pharmacology Laboratory of the Western Regional Research Laboratory of the United States Department of Agriculture. Booth *et al*[304], studied short term feeding of animals and showed that xanthan gum does not cause any acute toxicity or growth inhibiting

activity. Xanthan gum is nonsensitizing[349] and causes no eye or skin irritation. It also produced no mortality, no signs of toxicity or changes in the internal organs of rat or dogs given doses as high as 45g/kg and 20g/kg respectively. In long term (two years) feeding studies in rats and dogs and a reproduction study for three generations in the rat, it showed no significant effect on growth rate, survival, haematological values, or organ weight and no incidence of tumours[305].

The calorific availability test method shows that the digestibility of xanthan gum is zero. However more accurate radioactive tracer methods showed that the digestibility is approximately 15 percent. The approximate calorific value of xanthan gum is about 0.5 kcal g^{-1} (2.1kJ g^{-1}).

These feeding tests indicated that xanthan gum was safe for oral consumption and the Food and Drug Administration (FDA) issued a food additive order in 1969 that permitted the use of xanthan gum in food products without any specific quantity limitations[350].

In the United States, FDA regulations permit addition of xanthan gum to many standardized foods, such as cheeses,cheese products, milk and cream products, mellorine, food dressing, table syrups, vegetables in butter sauce, French dressing and other salad dressings. In addition the FDA has approved the use of xanthan gum as a suspension aid or stabilizer in the manufacture of paper and paper board intended for food contact.

The inclusion of xanthan gum in sauces, gravies and breading employed with meat and poultry products was also approved by USDA[351]. The use of xanthan gum alone as an inert ingredient in pesticide formulations and in combination with locust bean gum was allowed

by the United States Environmental Protection Agency (EPA) to be used on growing crops or raw agricultural commodities[352].

The general use of xanthan gum in foods was also formally approved by the Canadian Governor-in-Council[353]. Xanthan gum is included in Annexe II of the European Economic Community Emulsifier/Stabilizer list (EEC, 1974) and is designated as E415. The Joint Expert Committee of the Food and Agriculture Organization/World Health Organization (FAO/WHO) of the United Nations has issued an acceptable daily intake (ADI) or the product[354]. In addition, many other countries have approved xanthan gum for various food uses[355].

Food Applications:

Some of the food applications of xanthan gum have been mentioned above (for review see[307,344,355-357]) some specific food applications where xanthan gum has found utility are namely: bakery filling, dairy products, such as processed cheese spread[358], pourable dressings[359], sauces and gravies.

Additional examples of food applications in which xanthan gum has found use include canned foods, dry mixes, frozen foods, juice drinks, relishes, spoonable dressings and syrups. Related areas where the extraordinary supplements consist of nutrient materials that include vitamins and mineral salts suspended in molasses or water[357], and calf milk substitutes. In canned gravy-type pet foods, xanthan gum alone and in blend with galactomannans shows potential as a gravy thickener. These unique combinations of properties result from the high working yield value and high degree of pseudoplasticity of xanthan gum. Because of these unique properties xanthan gum has been widely accepted by the food industry as a multi-

purpose stabilizer, thickener and processing aid.

Industrial Applications:

As for food applications some of the industrial applications have been mentioned earlier. Summarizing, xanthan gum has been industrially used as:

abrasives, adhesives, agricultural (as a suspending agent for herbicides, pesticides, fertilizers, fungicides and agricultural spray and foams as xanthan gum alone and in combination with locust bean gum), ceramics, cleaners, polish, ink, paint, petroleum (as a viscosifier for drilling fluids), textile, wallpaper and welding rods. Xanthan gum alone or, with locust bean gum, also has been industrially sued as deodorant gels, fire fighting fluids, paper, blasting explosive (as a gel to produce water-resistant slurries) and finally photographic processing.

Carboxymethyl Cellulose (CMC)

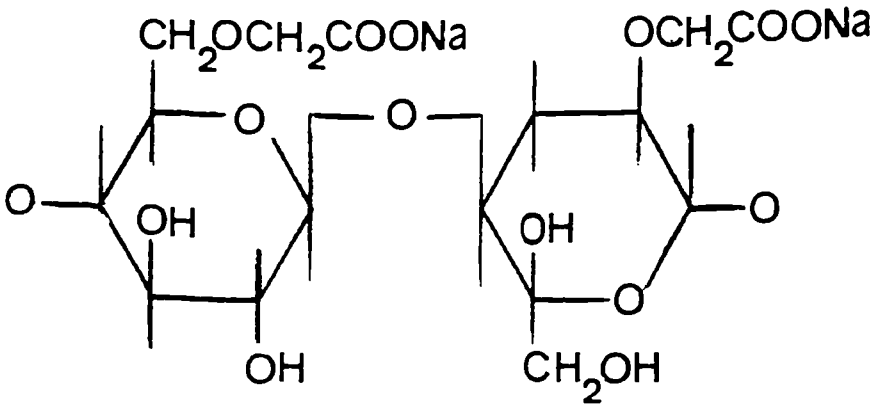
This anionic polysaccharide is a derivative of cellulose in which the hydroxyl groups have been substituted by carboxymethyl groups. CMC is usually used in the form of the sodium salt (NaCMC). The manufacture of NaCMC is a fairly simple, conventional chemical reaction. Purified cellulose is first treated with sodium hydroxide to swell the fibres and then reacted with sodium monochloroacetate ($CH_2ClCOONa$) according to the following equations[360].

$$R - OH + NaOH \rightarrow R - ONa + H_2O$$

$$R - ONa + CH_2 - Cl - COONa \rightarrow R - O - CH_2 - COONa + NaCl$$

Substitution can be at one or all of the hydroxyl groups on each of the D-glucose units of cellulose. The structure of a typical repeating unit is given in Figure 15[361].

Figure 15: Idealized structure of Na CMC.



The degree of substitution varies with industrial use. A degree of substitution of 1.0 refers to one carboxymethyl group per monomer, the C-6 position being preferentially substituted. Theoretically, three carboxy methyl groups per D-glucose unit can be introduced to the cellulose molecule with a resulting product having a degree of substitution of three.

NaCMC is soluble in water, displaying gum-like properties[362]. the optimum solubility and preferred physical properties are obtained with a much lower degree of substitution. In commercial products the degree of substitution ranges from 0.4 to 1.2 carboxymethyl groups per glucose unit and the average molecular weight ranges from approximately 50,000 to 500,000[361,363]. NaCMC is of wide use as a substitute for plant gums in industry, having many applications[364] and has also been found to have antitumour activity[365].

Crosslinked carboxymethyl cellulose (CLCMC) finds use as a valuable absorbent material. Bhattacharjee and Perline[366] have found that its absorbency and retention characteristics with respect to water and saline can be substantially improved by two different treatments.

One treatment involves radiation induced grafting of polystyrene onto fibres of CLCMC. A second means for enhancing the water and saline retention value of CLCMC, consists of subjecting the material to γ-irradiation from a ^{60}Co source. They concluded that the enhanced absorptivity of the CLCMC arises from partial breakdown of the fibres caused by γ-irradiation and a concomitant loosening of the crosslinked network. Therefore it may prove advantageous to sterilize absorbent preparations containing CLCMC by means of ^{60}Co irradiation[366].

CHAPTER THREE

MATERIALS AND METHODS

Experimental:

Material:

Alginates extracted from *Ascophyllum hodosum* (manucol DMF), *Macrocystic pyrifera* (manugel DMB) and *Lamineria kyperborea* (manugel DPB), were kindly provided by Dr D Lowe, Kelco International (London, England). Bronopol (an antibacterial agent) and glucono-δ-lactone were obtained from Johnson and Johnson and Sigma respectively. All of the other reagents used were of the highest grade available commercially.

Methods:

Determination of the Dose Rate Using the Fricke Dosimetry[367]:

The principle for this method for determining the dose received by a solution, is the oxidation of an aqueous aerated acid solution of ferrous sulphate to ferric sulphate:

$$Fe^{2+} + OH \rightarrow Fe^{3+} + OH^{-}$$

The degree of oxidation is linearly proportional to the radiation dose. The amount of ferric ion formed is determined spectrometrically, at 304nm (the maximum absorption of Fe^{3+} ions). The dosimeter solution ($500cm^3$) was prepared by dissolving ferrous sulphate (0.2g), sodium chloride (0.3g) and sulphuric acid (98%, $11cm^3$) in triply distilled water. The solution was air saturated and irradiated using ^{60}Co γ-irradiation for increasing times up to ten minutes. the increase in the Fe^{3+} ion concentration was measured at 304nm by measuring the increase in absorbance against an unirradiated solution as a blank.

From the graph of A_{304} V time the dose rate was calculated from the initial slope of the graph. The mean absorbed dose (Dm) was calculated as follows:

From the slope (S) and the Beer-Lambert Law, the number of moles of ferric ions produced per cm^{-3} is

$$= (S/E) \times 6.023 \times 10^{20}$$

where E = molar extinction coefficient of the ferric ion,

$$= 2125 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$$

Assuming a value of $G(Fe^{3+})$ = 15.5

Therefore the energy input is

$$= \frac{S \times 6.023 \times 10^{20} \times 100}{2125 \times 15.5} \text{ ev ml}^{-1} \text{ min}^{-1}$$

1 rad = 6.24×10^{13} ev ml^{-1}

Therefore, the dose at any absorbency is

$$= \frac{S \times 6.023 \times 10^{20} \times 100}{2125 \times 15.5 \times 6.24 \times 10^{13}}$$

The average dose was calculated in Gy/min.

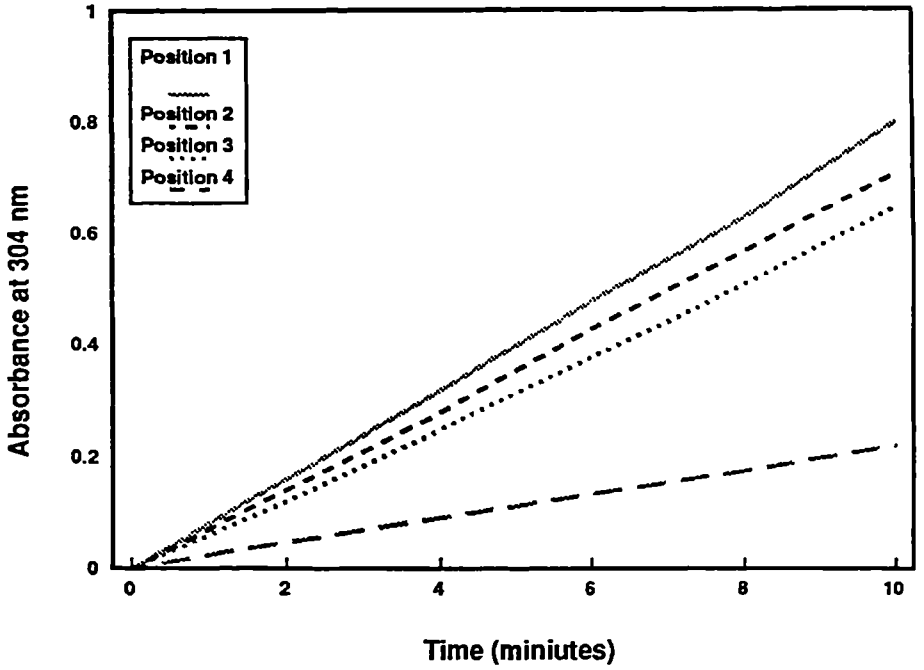
1Gy = 100 rad/or 1krad = 10Gy

The dose rates at four different positions around the ^{60}Co-γ-source are shown in Table XVII and are plotted in Figure 16.

Table XVII: Data for optical density (OD) at 304nm versus time (minutes) of irradiation using (Fricke dosimeter) for four positions around the ^{60}Co-γ-source.

Time (minutes)	Absorbance at 304nm			
	Position (1)	Position (2)	Position (3)	Position (4)
2	0.16	0.14	0.12	0.048
4	0.32	0.28	0.25	0.092
6	0.49	0.43	0.38	0.135
8	0.63	0.57	0.51	0.178
10	0.80	0.71	0.65	0.225
Dose rate (Gy/min)	23.38	20.86	19.04	6.51

Figure 16: Determination of the dose rate of a ^{60}Co-gamma-source using the Fricke dosimeter



Preparation of Alginate Solutions:

Solutions (2%) of three different types of Na-alginate namely, Manugel DMB, Manugel DPB and Manucol DMF were prepared at room temperature using a magnetic stirrer and an overhead stirrer. The water was first stirred at high speed and powdered alginates dusted slowly onto the vortex and a solution was obtained before thickening destroys the vortex.

As is common with most water-soluble hydrophillic colloids, alginates in a single granule will wet immediately and therefore when added to water dissolve easily. But when a mass of granules is added to water without sufficient agitation to complete dispersion, clumps of granules are formed. This is because the surfaces of the clumps solvate, forming a layer which prevents wetting to the interior of the clump. Therefore uniform dispersion of the algin powder in water is the key to rapid preparation of smooth solutions.

In all cases the alginate solutions were prepared on a percentage weight basis, as a 2% solution by dissolving 2g of alginate powder in $100cm^3$ distilled water containing 0.02g bronopol as an antibacterial.

When mannitol and ascorbate were to be included in the alginate solution, they were both dissolved in water prior to addition of sodium alginate powder.

Thickening or Gel Formation of Sodium Alginate Solutions:

The 2% alginate solution of different type of algin were thickened using three different methods.

Method 1

A pumpable jelly suitable for use as a glaze/topping (specially with manucol DMF). This jelly is thixotropic. Preparation was in two parts as follows:

Part 1

	%	
Sugar	37.60	g
Sodium alginate	0.7	g
Sodium hexametaphosphate	0.12	g
Dicalcium phosphate dihydrate	0.07	g

These were mixed in a dry state, added to 56.36cm^3 of deionised water and stirred with good agitation until a lump free, homogenous solution is obtained. The solution was heated to 70°C.

Part 2

Citric acid (0.15g) was dissolved in deionised water (5cm^3). This solution (part two) was added to part one (total volume of 100cm^3) and mixed for one minute. The mixture was immediately cooled to 10°C to 20°C in ice and a gel formed on cooling.

The method was modified by adding the appropriate volume of irradiated alginate solutions (with or without mannitol) to a mixture instead of the dry powder. A known volume of solution was poured into Petri dishes to make a gel.

Method 2

The following formulation gives a gel which is demouldable after 5-10 minutes. Suitable for manugel DPB.

This method also is in two parts and contains the % of the following materials:

Part 1

	%	
Deionised water	90.11	cm^3
Alginate	0.4	g
Sodium citrate dihydrate	0.07	g
Disodium orthophosphate	0.02	g
Dicalcium phosphate, anhydrous	0.18	g

Part 2

Deionised water	9.15	cm^3
Citric acid	0.07	cm^3

Parts 1 and 2 were made separately and then part 2 was added to part 1 and stirred by hand for 10 to 15 seconds. As in method one in the case of irradiated alginate solutions (with or without mannitol) an appropriate volume of alginate solutions were used in the mixture. Known volumes were poured into Petri dishes to set as a thick solution or gel within 5-10 minutes.

Method 3

This method was finally used for making gels throughout this study. Solutions of three

sodium alginates were prepared as described earlier to a final concentration of 2%. Calcium orthophosphate (0.5g) was slurried in water ($10cm^3$) and mixed with the sodium alginate solution (with or without mannitol). Gluconolactone (0.5g) was dissolved in water ($10cm^3$), added to the mixtures and the resultant solution was mixed thoroughly. Known volumes were poured into Petri dishes. A fibre support (an open nylon mesh) was pushed through the solution in the Petri dish and the preparation was left to gel (~ 10-15 mins), then covered to prevent evaporation and the gels from drying. In some experiments different amounts of calcium orthophosphate and gluconoluctone were used.

Gels of different final alginate concentrations were prepared by evaporating 2% gels to various extents in a vacuum oven at 50°C. Gels were prepared containing 4%, 6% and 8% alginate by evaporation to half, one third and a quarter of the initial gel weight.

γ-Irradiation of Alginate Solutions

The three different alginates described before were irradiated at the ^{60}Co-γ-source situated at Salford University. Bulk solutions were pre-gassed by bubbling with air or nitrogen during preparation and then divided up into smaller portions in sealed bottles. The bottles were irradiated using a ^{60}Co-γ-ray source up to a dose of 32kGy which is above the terminal sterilisation dose. Control or zero dose samples were treated as for all other samples but were not exposed to irradiation.

In this part of the study, the alginate solutions were thickened using the three methods described earlier, before and after irradiation of the alginate solutions. In these instances the sodium alginate solutions were prepared with or without mannitol as above, irradiated and then thickening agents were added to the irradiated solutions previously described.

Measurement of Viscosity

The viscosities of sodium alginate solutions at zero dose and irradiated to different doses were measured using a Brookfield L.V.T. dial viscometer at different spindle speeds (rpm) and spindles as described earlier. Measurements were made at 20°C. The viscosity of the thickened samples were measured. In some instances however gel formed. In this case they were first cut into small pieces and blended for 5 minutes prior to measurement of viscosity.

γ-Irradiation of Alginate Gels

Each gel prepared by method 3 was weighed accurately and packed in an air tight plastic bag. Each bag was mounted vertically around the ^{60}Co-γ-source and irradiated to increasing doses up to 32kGy. Dosimetry was carried out in sealed Petri dishes mounted vertically using the Fricke dosimetry. In some experiments both the alginate solutions and the calcium orthophosphate and gluconolactone (the gelling agents) were irradiated before preparation of the gel, i.e. the components of the gel were irradiated prior to preparation of the gel.

Water Release

After irradiation the amount of water released from the gels (due to irradiation) was determined by re-weighting the gel, the difference between this weight and the pre-irradiation weight was assumed to be the water loss.

Water and Saline Uptake

Weighed gels were immersed in small baths containing distilled water or 0.15 mol dm^{-3} saline solution, before and after irradiation for increasing times. They were removed, blotted dry and re-weighed. Changes in gel weight were then taken to be due to water or saline uptake.

Gel Strength

This was performed by fixing the gel on two plastic plates connected by a hinge. The angle of rupture was read on a protractor attached to one of the plates as shown in the photograph.

Xanthan Gum Study:

Materials:

Xanthan gum as KETROL T (transparent) Batch No 51015V and locust bean gum (LBG) were provided by Kelco Division of Merck and Co. Inc. All other chemicals were reagent grades used without further purification.

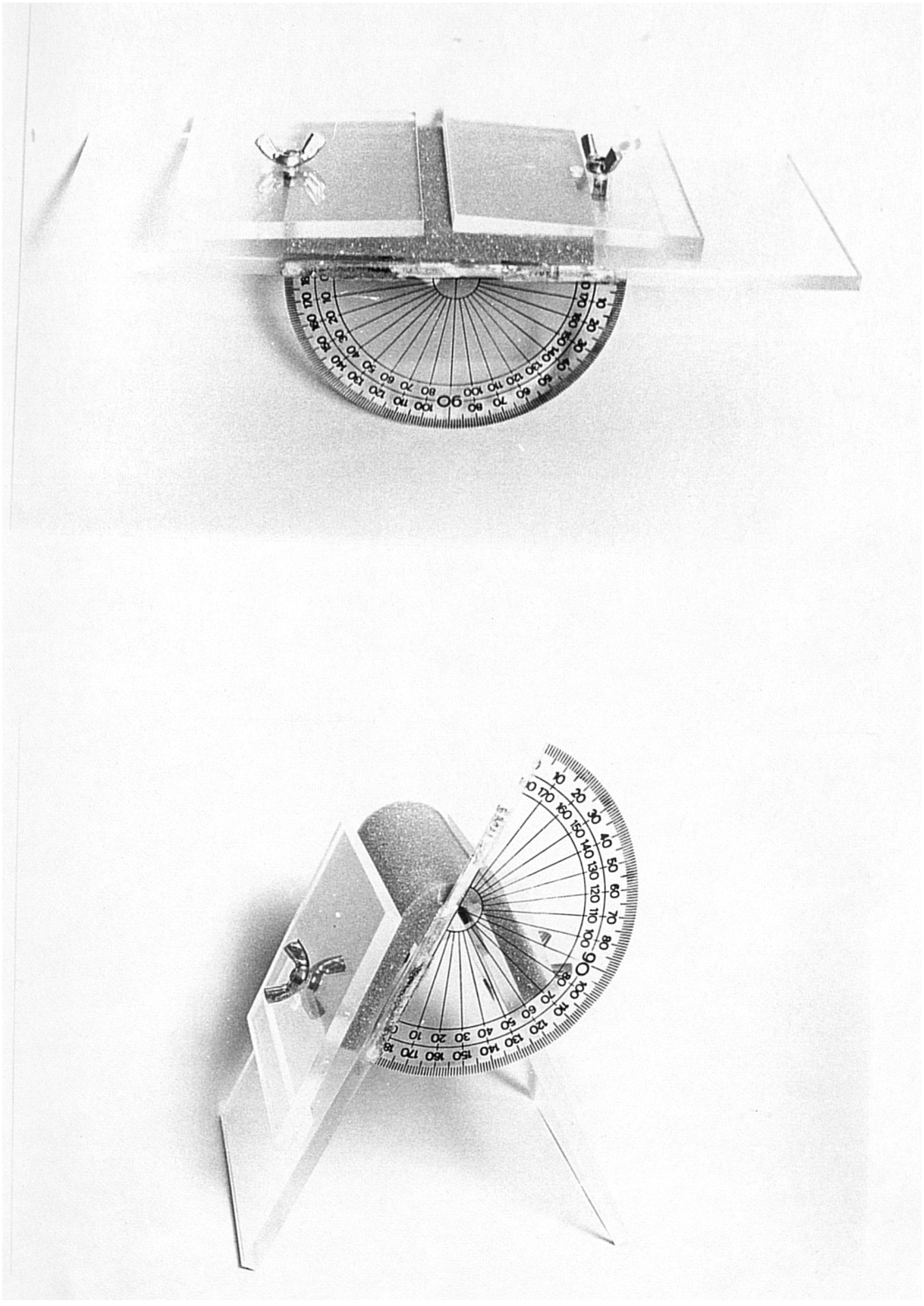
Methods:

Preparation of Xanthan Gum Solutions:

A 1% solution of Keltrol was prepared by bubbling 99 cm^3 of deionized water with air and adding with stirring 1.0 gram of dry blend Keltrol slowly onto the upper wall of the vortex so that the individual granules are wetted out. The addition should be competed before thickening destroys the vortex. It was then put in a water bath at 25°C and stirred vigorously by hand to eliminate any possible lumps. When mannitol, ascorbic acid and NaCl were to be added in the xanthan gum, they were dissolved in water prior to addition of xanthan gum or LBG powder.

Thickening or Gel Formation of Xanthan Gum Solutions:

1% xanthan gum solutions were thickened by addition of 1% LBG solution (1:1). LBG solutions (1%) were made in a similar way to xanthan gum to produce a smooth solution with no lumps.



Viscosity Measurements:

Viscosities were measured at 20°C using a Brookfield Digital Viscometer Model DV-II with a small sample adaptor and a two speed spindle SC4-18/13R and SC4-25/13R at a shear rate of 1.32N and 0.22N s^{-1} (N=RPM) respectively. In all viscosity measurements 8 speeds were used namely, 0.3, 0.6, 1.5, 3, 6, 12, 30 and 60 RPM.

Thickened solutions were similarly measured. In some instances however a gel formed. In this case they were smashed or blended as described for alginate gels, prior to viscosity measurements.

γ-Irradiation of Xanthan Gum Solutions:

Xanthan gum solutions (1%) with or without mannitol (20%) and mannitol/ascorbic acid (10^{-2} mol dm^{-3}) were irradiated. Bulk solutions were pregassed by bubbling with air, N_2 and N_2O, during their preparation and then irradiated as aliquots under these gasses up to increasing doses of ~27kGy.

Xanthan gum solutions containing LBG (1:1) were irradiated as for the above samples.

Carboxymethyl Cellulose (CMC) Study:

Materials:

Sodium carboxymethyl cellulose USP 7mF and sodium carboxymethyl cellulose USP 7HCF were obtained from Hercules Company Limited, London, U.K.

Methods:

Preparation of CMC Solutions:

A solution of 3.5% CMC (100 cm^3) was prepared by mixing CMC USP 7MF (2.9g) and CMC USP 7HCF (0.6g). When mannitol or mannitol ascorbic acid was included in the CMC solutions, they were both dissolved in water prior to addition of CMC solutions.

γ-Irradiation of CMC:

All solutions were pregassed with air or N_2, sealed and aliquots, irradiated for increasing doses up to 27kGy.

Viscosity Measurements:

The viscosity of CMC solutions both irradiated and non-irradiated were measured using a Brookfield LVT viscometer using spindle numbers 18 and 25 and a range of shear rates.

CHAPTER FOUR

RESULTS

RESULTS

Sodium Alginate Solutions:

The flow properties of sodium alginate solutions are dependent on the concentration of the alginate. At concentrations of $\geq 2\%$ the extent of shear thinning increases and such solutions are said to be non-Newtonian. For viscometric measurements of non-Newtonian fluids to be meaningful, the measurements must be determined over a range of shear rates, because the apparent viscosity of these solutions various with shear rate. This is demonstrated for various alginate solutions in Figure 17. The apparent viscosity of Manugel DMB, Manugel DPB and Manucol DMF decreases as the spindle speed (which is proportional to shear rate) increases. For example as the spindle speed increases from 0.6 to 60rpm the apparent viscosity of DPB decreases from 6000 cps to ~3000 cps (ie. almost 50%). Solutions of 2% DMB and DMF have similar apparent viscosities over the same range of spindle speed as above for DPB. Their apparent viscosities are, however, considerably less than that for DPB and again decreases, albeit to a lesser extent as the spindle speed increases. All of the solutions are non-Newtonian and are also said to be thixotropic and pseudoplastic (shear thinning).

Addition of mannitol (15%) to DMB, DPB and DMF solutions (all 2%) results in an increase in the apparent viscosity of all spindle speeds (Figure 18) though the same decrease in apparent viscosity occurs with increasing spindle speed as was previously observed, indicating that alginate/mannitol solutions are also thixotropic and pseudoplastic.

Solutions (2%) of DMB, DPB and DMF were irradiated in N_2 and air to increasing doses (up to 25kGy) and their apparent viscosity was again measured over the range of spindle speed of 0.6-60rpm. The results are given in tables XVIII-XX. The machine (Brookfield

Figure 17: Relationship between apparent viscosity and spindle speed (RPM) for three different unirradiated sodium alginate solutions (2%). Using a Brookfield L.V.T. viscometer and the appropriate spindle. Temperature = 20°C

(A): As a bar
(B): As a line

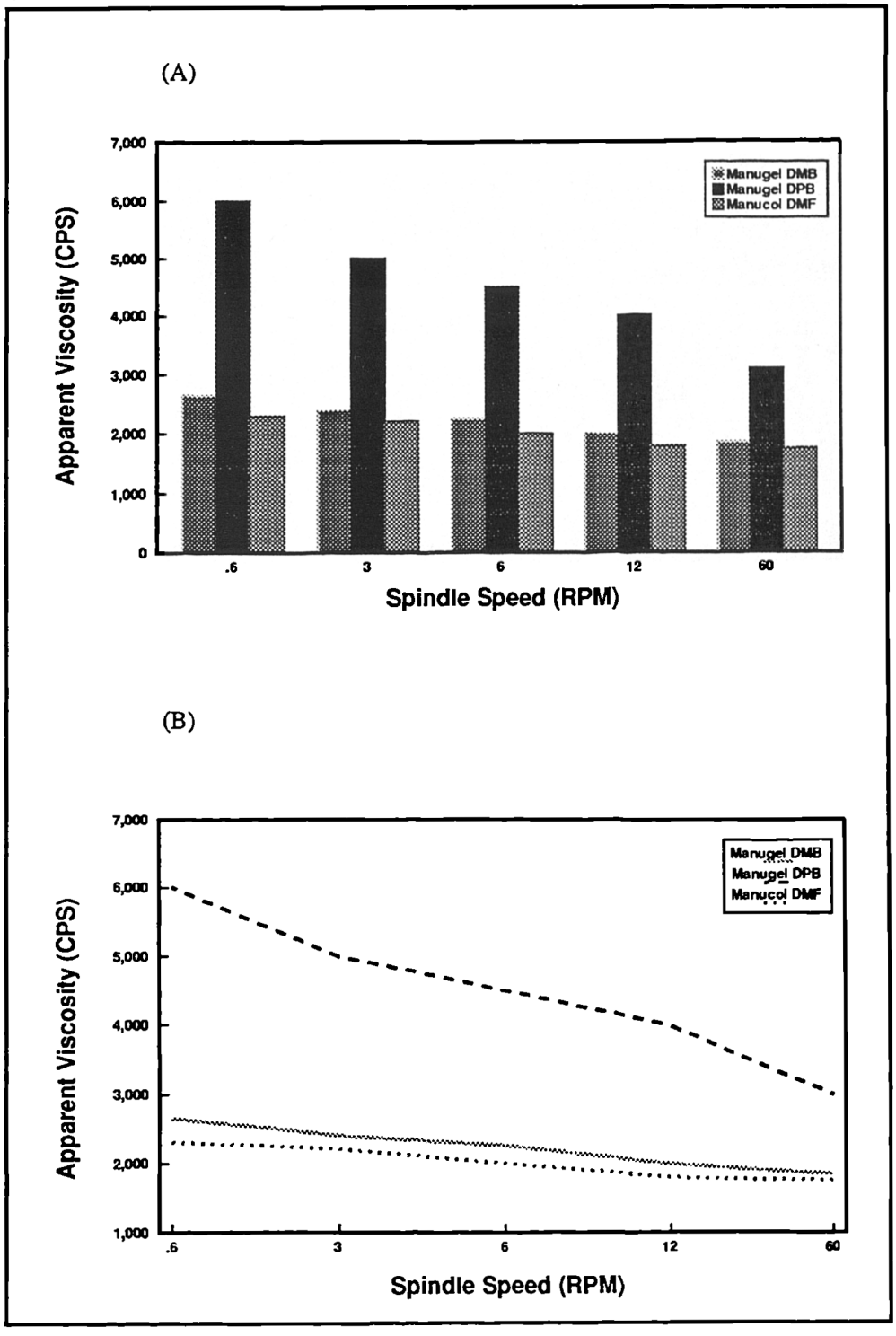


Figure 18: Relationship between apparent viscosity and spindle speed (RPM) for three different unirradiated sodium alginate solutions (2%) containing mannitol (15%). Using a Brookfield L.V.T. viscometer and the appropriate spindle. Temperature = 20°C

(A): As a bar
(B): As a line

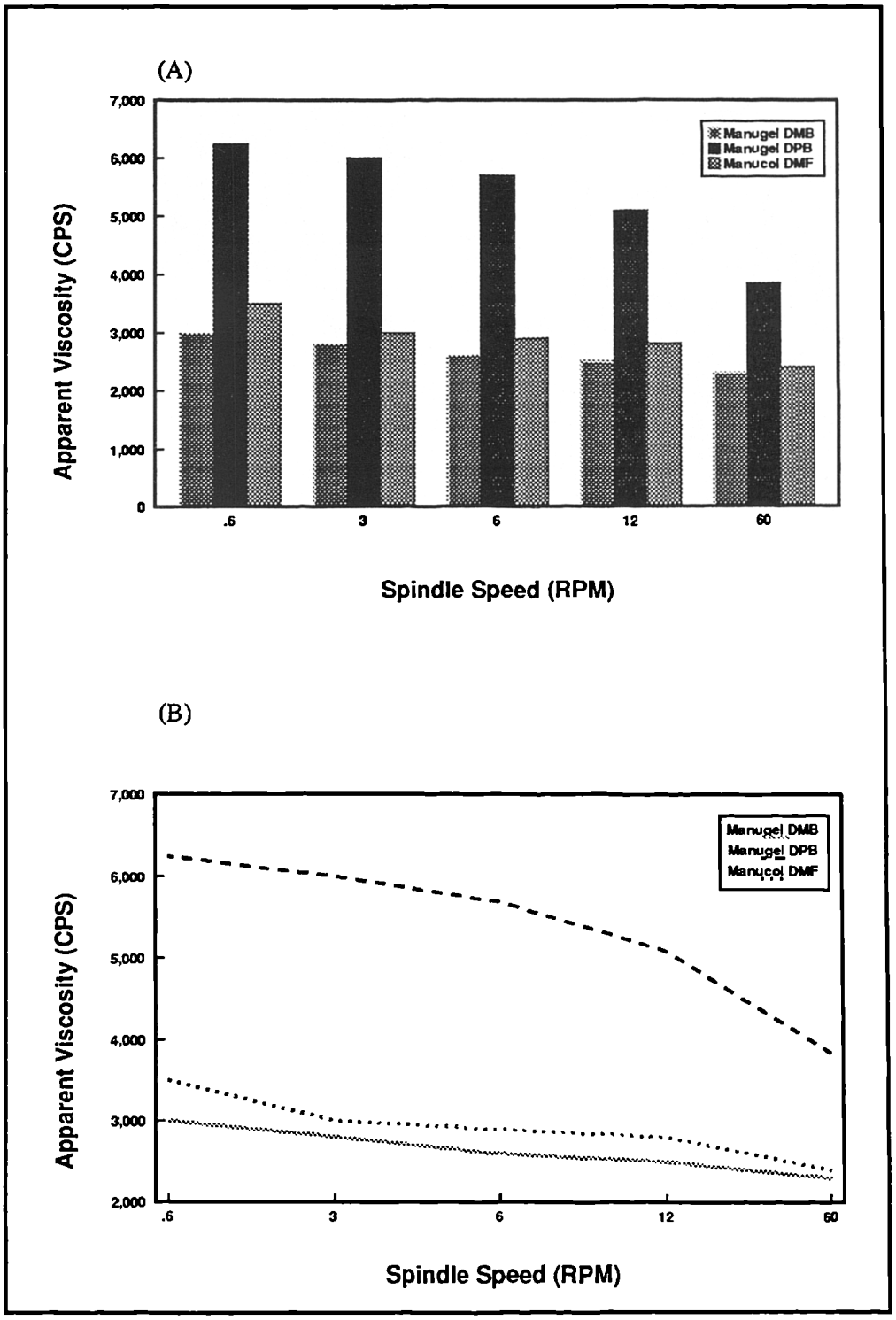


Table XVIII: Determination of the apparent viscosity of manugel DMB (2%), using a Brookfield L.V.T. viscometer. Temperature = 20°C.

Apparent Viscosity (CPS)

Spindle	Dose (kGy)	0		0.25		0.5		0.75		1.0		2.0		3.0	
	Speed (rpm)	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
1	0.6	2650	2650	<10	1050	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	>100*	>100	450	950	<10	500	<10	360	<10	240	<10	<10	<10	<10
	6	>100	>100	445	>100	145	450	<10	350	<10	235	<10	90	<10	<10
	12	>100	>100	440	>100	147.5	430	82.5	330	47.5	222.5	<10	87.5	<10	<10
	60	>100	>100	>100	>100	>100	>100	80.5	>100	48	>100	18	86.5	13.5	31.5
2	0.6	2500	2500	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	2100	2400	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	2200	2150	450	850	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	2125	2050	450	800	<10	400	<10	262.5	<10	<10	<10	<10	<10	<10
	60	>100	>100	442.5	>100	150	387.5	75.0	255	47.5	182.5	<10	80	<10	<10
3	0.6	<10*	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	2200	2250	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	2200	2000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	1800	1740	500	780	<10	410	<10	250	<10	180	<10	<10	<10	<10
4	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	2000	1850	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

* See text

Table XIX: Determination of the apparent viscosity of manugel DPB (2%) using a Brookfield L.V.T. viscometer. Temperature = 20°C.

Apparent Viscosity (CPS)

Spindle	Dose (kGy)	0		0.25		0.5		0.75		1.0		2.0		3.0	
	Speed (rpm)	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
1	0.6	7750	>100*	2000	3200	<10	1150	<10	<10	<10	<10	<10	<10	<10	<10
	3	>100	>100	>100	>100	800	1100	<10	550	260	320	<10	<10	<10	<10
	6	>100	>100	>100	>100	795	>100	465	520	250	310	<10	<10	<10	<10
	12	>100	>100	>100	>100	>100	>100	>100	>100	250	300	80	<10	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	79.5	70	31	36
2	0.6	7500	6250	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	5550	6000	1800	2800	<10	1100	<10	<10	<10	<10	<10	<10	<10	<10
	6	>100	>100	1800	2650	725	1075	<10	500	<10	<10	<10	<10	<10	<10
	12	>100	>100	1800	>100	725	987.5	400	500	<10	312.5	<10	<10	<10	<10
	60	>100	>100	>100	>100	>100	>100	400	475	230	282.5	75	65	<10	<10
3	0.6	<10*	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	6000	6000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	5600	5700	1800	2600	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	5100	5200	1800	2400	<10	1050	<10	<10	<10	<10	<10	<10	<10	<10
	60	>100	>100	1570	>100	800	900	410	470	220	280	<10	<10	<10	<10
4	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	5250	5100	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	4200	3850	1700	2150	<10	1000	<10	<10	<10	<10	<10	<10	<10	<10

* See text

Table XX: Determination of the apparent viscosity of manucol DMF (2%), using a Brookfield L.V.T. Viscometer. Temperature = 20°C.

Apparent Viscosity (CPS)

Spindle	Dose (kGy)	0		0.25		0.5		0.75		1.0		2.0		3.0	
	Speed (rpm)	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
1	0.6	2950	2300	1100	850	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	>100*	>100	1000	840	500	370	300	300	200	<10	<10	<10	<10	<10
	6	>100	>100	>100	830	420	350	280	280	195	230	<10	<10	<10	<10
	12	>100	>100	>100	>100	400	340	260	270	180	220	<10	<10	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	36	55	20	20
2	0.6	<10*	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	2650	2200	1100	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	2600	2150	1075	800	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	>100	2100	1000	775	450	350	262.5	250	<10	225	<10	<10	<10	<10
	60	>100	>100	>100	>100	440	340	260	242.5	175	207.5	50	50	<10	<10
3	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	2600	2000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	2500	1800	1000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	>100	1760	960	780	440	340	260	240	<10	200	<10	<10	<10	<10
4	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	2300	1900	1000	750	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

* See text

L.V.T. viscometer) measures viscosities when the values of the torque are between 10 and 100. Therefore the terms >100 and <10 have been used to indicate that for a particular spindle certain speeds cannot be used to determine the apparent viscosity. For example in table XVIII, for unirradiated DMB using spindle 1, the only appropriate spindle speed is 0.6rpm, whereas using spindle 4 the only appropriate speed is 60rpm. For spindles 2 and 3, the apparent viscosity can be measured using speeds of 0.6, 3, 6 and 12 rpm and 6, 12 and 60rpm respectively. Examination of the data as a whole indicates that the only spindle speed that can be used to determine the apparent viscosity over the entire range of radiation dose used is 60rpm. The apparent viscosity in air using spindle 4 (speed 60rpm) is 1850 cps. At 0.25 kGy, only spindle 3 can be used (apparent viscosity 780 cps), at 0.5kGy spindles 2 and 3 can be used (apparent viscosity 387.5 and 410 cps respectively), at 0.75 kGy again spindles 2 and 3 can be used (apparent viscosity 255 and 250cps respectively), at 1.0kGy spindles 2 and 3 give apparent viscosities of 182.5 and 180 respectively, at 2.0kGy spindles 1 and 2 give apparent viscosities of 86.5 and 80cps, at 3.0kGy only spindle 1 can be used which gives an apparent viscosity of 31.5cps. Above this dose no viscosities can be measured. The decrease in the apparent viscosity at increasing radiation dose is therefore only expressed graphically using a spindle speed of 60rpm. The data for DMB, DPB and DMF, provided in tables XVIII-XX are plotted in Figures 19 and 20. For all solutions the apparent viscosity decreased with increasing radiation dose.

DPB has the highest initial viscosity, though after a dose of only 2kGy in air and N_2 all solutions are extensively degraded and their viscosities are all less than 100cps. The extent of decomposition is generally greater in N_2 though the initial rate of decomposition of DMF in N_2 and air are approximately the same. At doses of >2kGy the viscosities are very small and are similar for all the alginate solutions irradiated (Figure 21).

Figure 19: Effect of γ-irradiation in air on the apparent viscosity of three different irradiated sodium alginate solutions (2%). Using a Brookfield L.V.T. viscometer at 60 rpm and the appropriate spindle. Temperature = 20°C.

(A): As a bar
(B): As a line

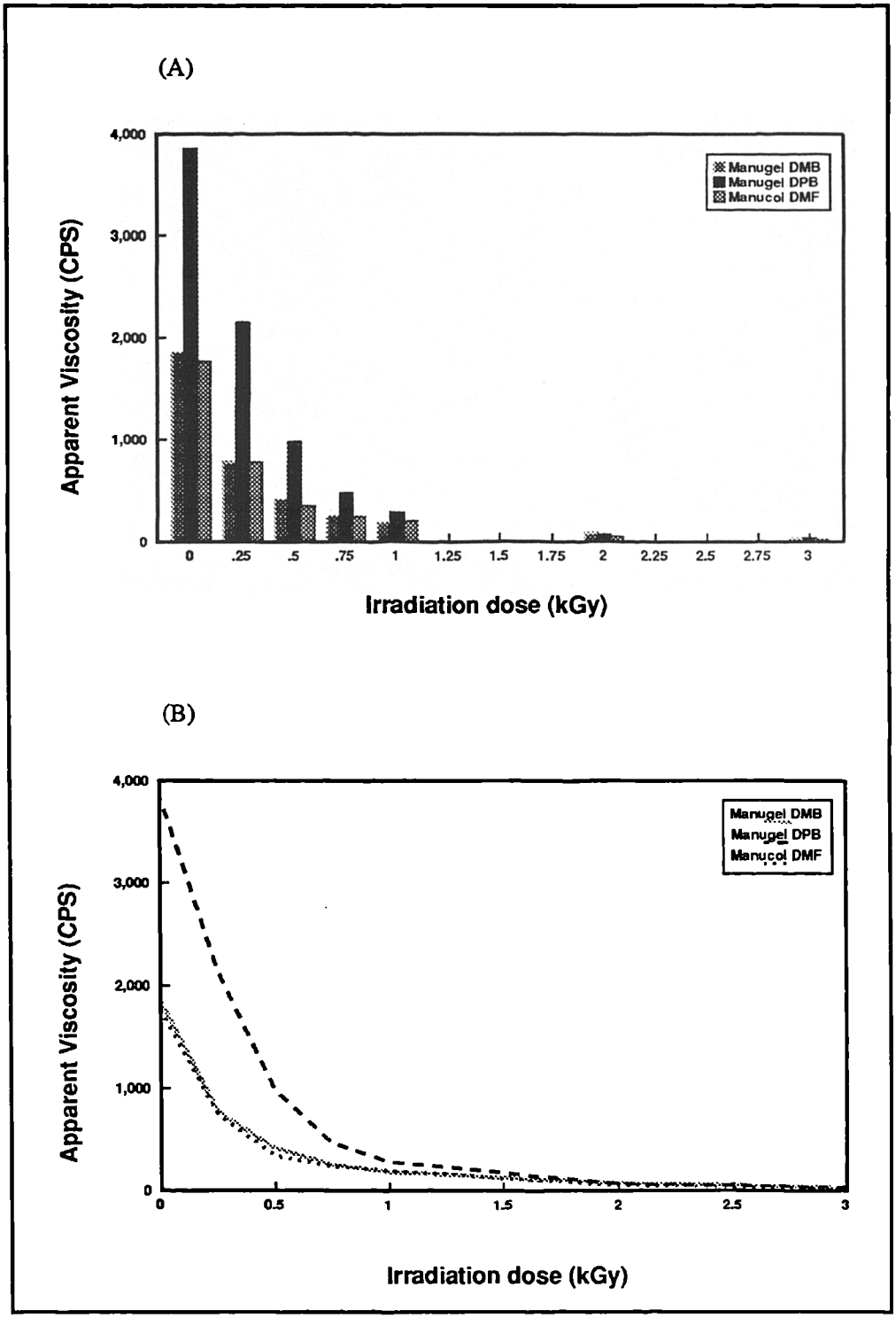


Figure 20: Effect of γ-irradiation in N_2 on the apparent viscosity of three different irradiated sodium alginate solutions (2%). Using a Brookfield L.V.T. viscometer at 60 rpm and the appropriate spindle. Temperature = 20°C.

(A): As a bar
(B): As a line

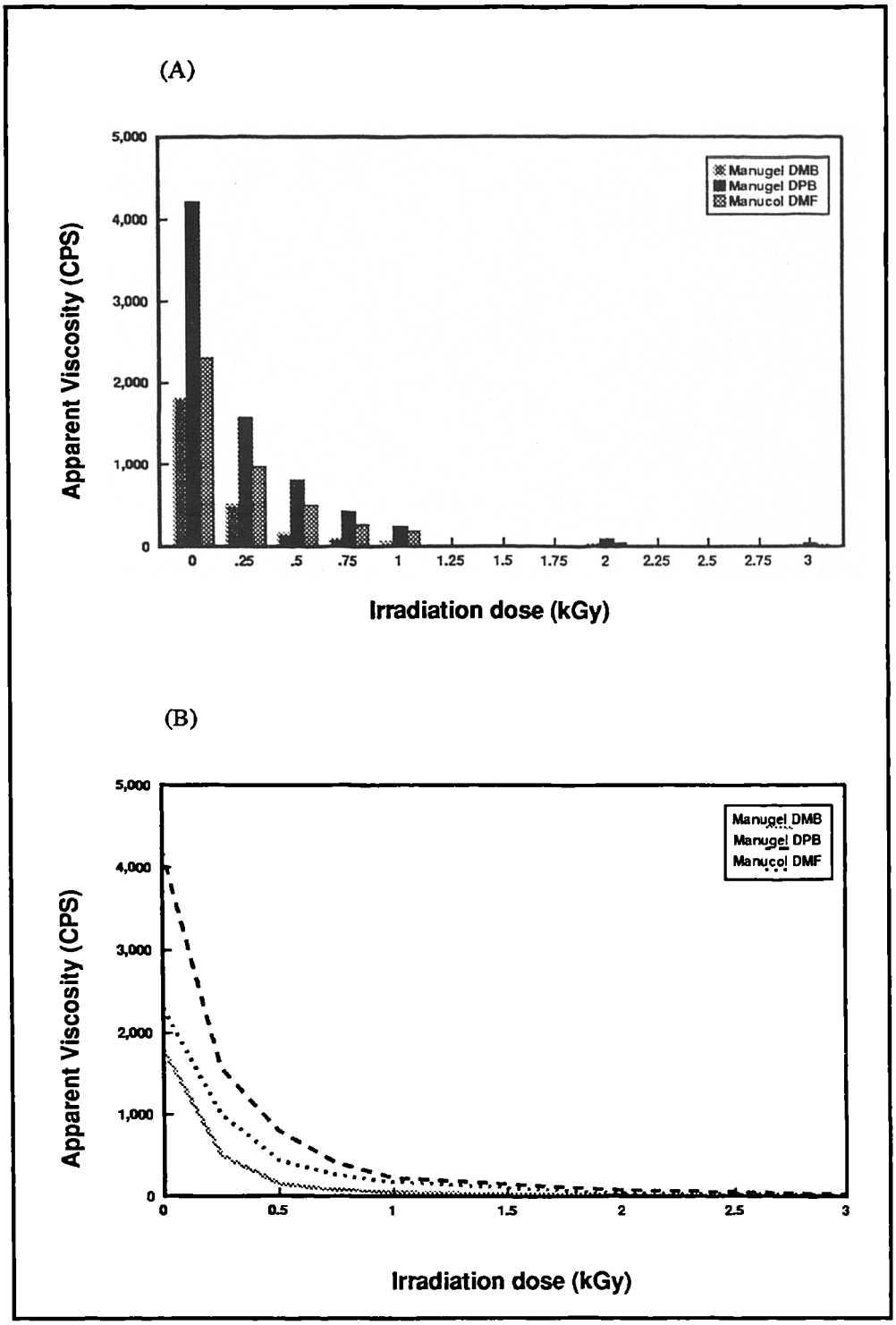
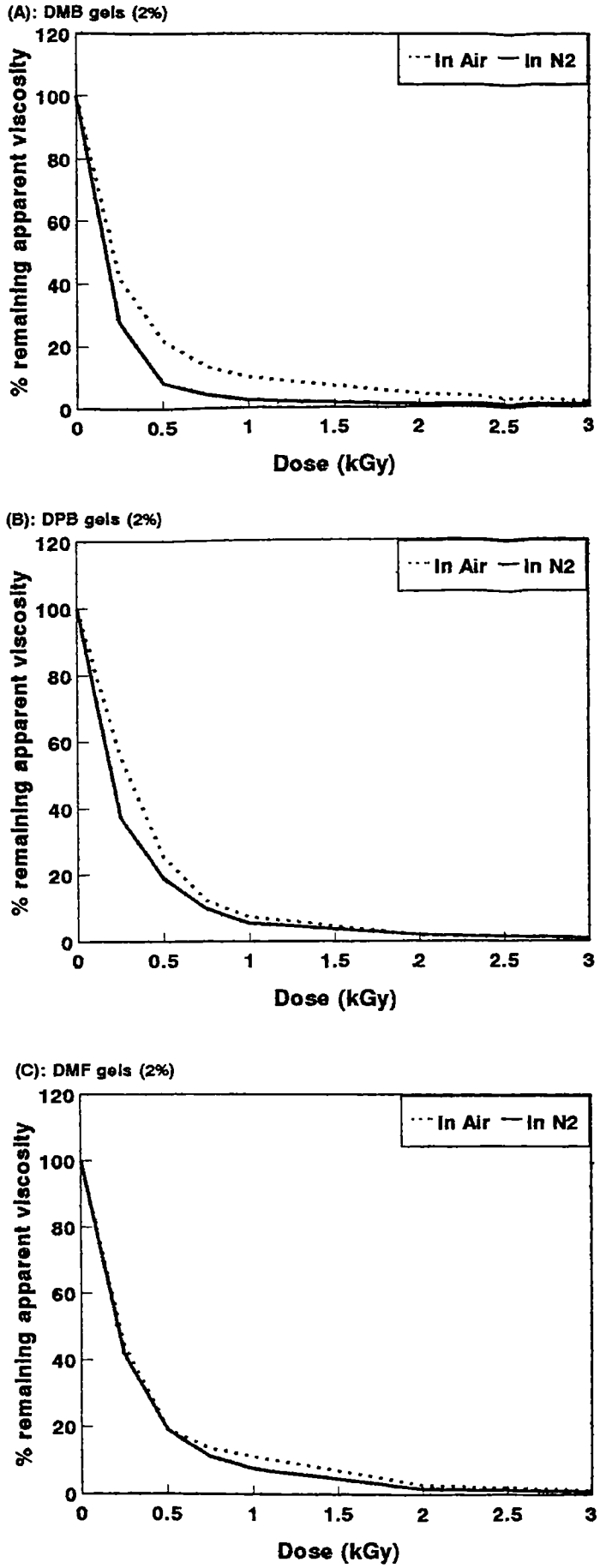


Figure 21: % remaining apparent viscosity for 2% alginate solutions irradiated in air and N_2



Radiolysis of alginates in the presence of mannitol results in some stabilisation of the solutions. Viscosities for all of the solutions can be measured up to doses of 25kGy (Tables XXI-XXIII). Figures 22-23, expresses the data up to 3kGy as for the previous solutions in the absence of mannitol. Again the solutions are initially non-Newtonian at doses of ~ 0.5 kGy but at this dose the solutions became Newtonian, particularly at doses of >2kGy.

No substantial differences are observed if the irradiation is carried out in air or N_2 (Figure 24).

Thickening of Alginate Solutions

The three methods used to thicken sodium alginate solutions were of varying effectiveness. Method 3, which used calcium orthophosphate and glucono-δ-lactone was the most effective in thickening the solutions, though all methods were reasonably effective (Table XXIV-XXVI). Method 1, which is used for pumpable commercial jelly preparations is thixotropic and will flow when sheared. Method 2 which is demouldable after 5-10mins gave gels of very high viscosity. Methods 1 and 2 yield better gels using DMF compared with DMB and DPB. When Method 3 is used to prepare gels all the alginate preparations gelled within approximately 10-15 minutes.

The three methods were used in all subsequent experiments, with the objective of trying to thicken those solutions that had been irradiated to sterilisation dose (25kGy). Method 3 produced solutions with reasonably high viscosity after 5kGy, for DMB, DPB and DMF, but none could be thickened after 25kGy (Table XXIV-XXVI).

Alginate solutions irradiated in the presence of mannitol to doses up to 25kGy can be

Table XXI: Determination of the apparent viscosity of manugel DMB (2%) containing mannitol (15%), using a Brookfield L.V.T. viscometer. Temperature = 20°C.

Apparent Viscosity (CPS)

Spindle	Dose (kGy)	0		0.25		0.5		0.75		1.0		2.0		3.0		5.0		25	
	Speed (rpm)	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
1	0.6	2500	3000	2200	2800	1700	2250	1400	2400	950	1700	900	<10	<10	<10	<10	<10	<10	<10
	3	>100*	>100	>100	>100	1600	>100	1400	>100	960	10	910	810	<10	610	<10	300	<10	<10
	6	>100	>100	>100	>100	>100	>100	>100	>100	965	>100	910	805	300	610	165	300	<10	<10
	12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	300	>100	167.5	300	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	20	38
2	0.6	<10*	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	2400	2800	2000	2150	1600	1850	1250	1850	950	1600	<10	<10	<10	<10	<10	<10	<10	<10
	6	2350	2750	2000	2125	1550	1870	1250	1850	950	1550	825	725	<10	550	<10	<10	<10	<10
	12	2250	>100	1875	2125	1500	1875	1250	1850	950	1550	825	725	275	550	<10	<10	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	285	>100	150	160	<10	<10
3	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	2300	2600	1800	2100	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	2400	2500	1850	2100	1400	1850	1250	1500	950	1350	<10	<10	<10	<10	<10	<10	<10	<10
	60	>100	>100	1530	1780	1360	1600	1200	1550	910	1100	600	710	290	530	<10	<10	<10	<10
4	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	2200	2300	1750	1900	1400	1500	1200	1300	<10	1350	800	<10	<10	<10	<10	<10	<10	<10

* See text

Table XXII: Determination of the apparent viscosity of manugel DPB (2%), containing mannitol (15%), using a Brookfield L.V.T. viscometer. Temperature = 20°C.

Apparent Viscosity (CPS)

Spindle	Dose (kGy)	0		0.25		0.5		0.75		1.0		2.0		3.0		5.0		25	
	Speed (rpm)	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
1	0.6	>100*	6000	8800	4250	6450	2900	5300	2400	4150	1900	1900	900	1000	<10	<10	<10	<10	<10
	3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	900	940	560	380	200	<10	<10
	6	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	900	935	540	380	200	<10	<10
	12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	380	190	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	16	12
2	0.6	10,000	5250	7000	<10	5500	<10	4500	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	7000	5000	>100	3000	5000	2500	4050	2000	3400	1700	1800	<10	1000	<10	<10	<10	<10	<10
	6	>100	4300	>100	2900	4900	2400	4025	2000	3300	1675	1750	800	1000	550	<10	<10	<10	<10
	12	>100	>100	>100	>100	>100	>100	>100	1875	>100	1550	1750	775	1000	500	350	<10	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	350	185	<10	<10
3	0.6	10,000	>100	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	7200	4600	6000	<10	5200	<10	4000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	6800	4500	6200	3000	5100	2400	4100	1900	3500	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	6000	4000	6000	2800	5100	2250	4100	1800	3400	1500	1600	<10	1000	<10	<10	<10	<10	<10
	60	>100	>100	>100	>100	>100	1820	>100	1600	>100	1390	1470	800	960	550	380	180	<10	<10
4	0.6	<10*	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	6500	<10	<10	<10	5000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	5000	3100	4200	2350	3700	2000	3200	1750	2600	1500	1550	700	1000	<10	<10	<10	<10	<10

* See text

Table XXIII: Determination of the apparent viscosity of manucol DMF (2%), containing mannitol (15%), using a Brookfield L.V.T. viscometer. Temperature = 20°C.

Apparent Viscosity (CPS)

Spindle	Dose (kGy)	0		0.25		0.5		0.75		1.0		2.0		3.0		5.0		25	
	Speed (rpm)	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air	N_2	Air
1	0.6	6250	3500	5100	3000	4400	2500	4100	2100	2100	1800	1450	1000	<10	<10	<10	<10	<10	<10
	3	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	1440	980	800	600	460	240	<10	<10
	6	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	960	730	590	400	220	<10	<10
	12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	390	215	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	15	15
2	0.6	2750	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	4500	3000	3500	2500	2750	1950	2300	1700	2000	1600	<10	1000	<10	<10	<10	<10	<10	<10
	6	407.5	2900	3575	2500	2650	1925	2200	1650	1800	1550	1100	950	650	525	<10	<10	<10	<10
	12	>100	>100	>100	>100	>100	1850	2150	1600	1750	1500	1000	850	625	500	350	<10	<10	<10
	60	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	325	207.5	<10	<10
3	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	4400	<10	4000	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	4200	3000	3600	2400	2600	2000	2200	<10	1800	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	4000	2800	3400	2000	2400	1850	2000	1500	1600	1500	1200	<10	<10	<10	<10	<10	<10	<10
	60	>100	>100	>100	1940	>100	1620	1860	1440	1560	1430	1100	900	640	530	340	220	<10	<10
4	0.6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	3	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	6	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	12	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
	60	3300	2400	2800	2150	2300	1750	2000	1500	1650	1250	1100	750	<10	<10	<10	<10	<10	<10

* See text

Figure 22: Effect of γ-irradiation in air on the apparent viscosity of three different irradiated sodium alginate solutions (2%), containing mannitol (15%). Using a Brookfield L.V.T. viscometer at 60 rpm and the appropriate spindle. Temperature = 20°C.

(A): As a bar
(B): As a line

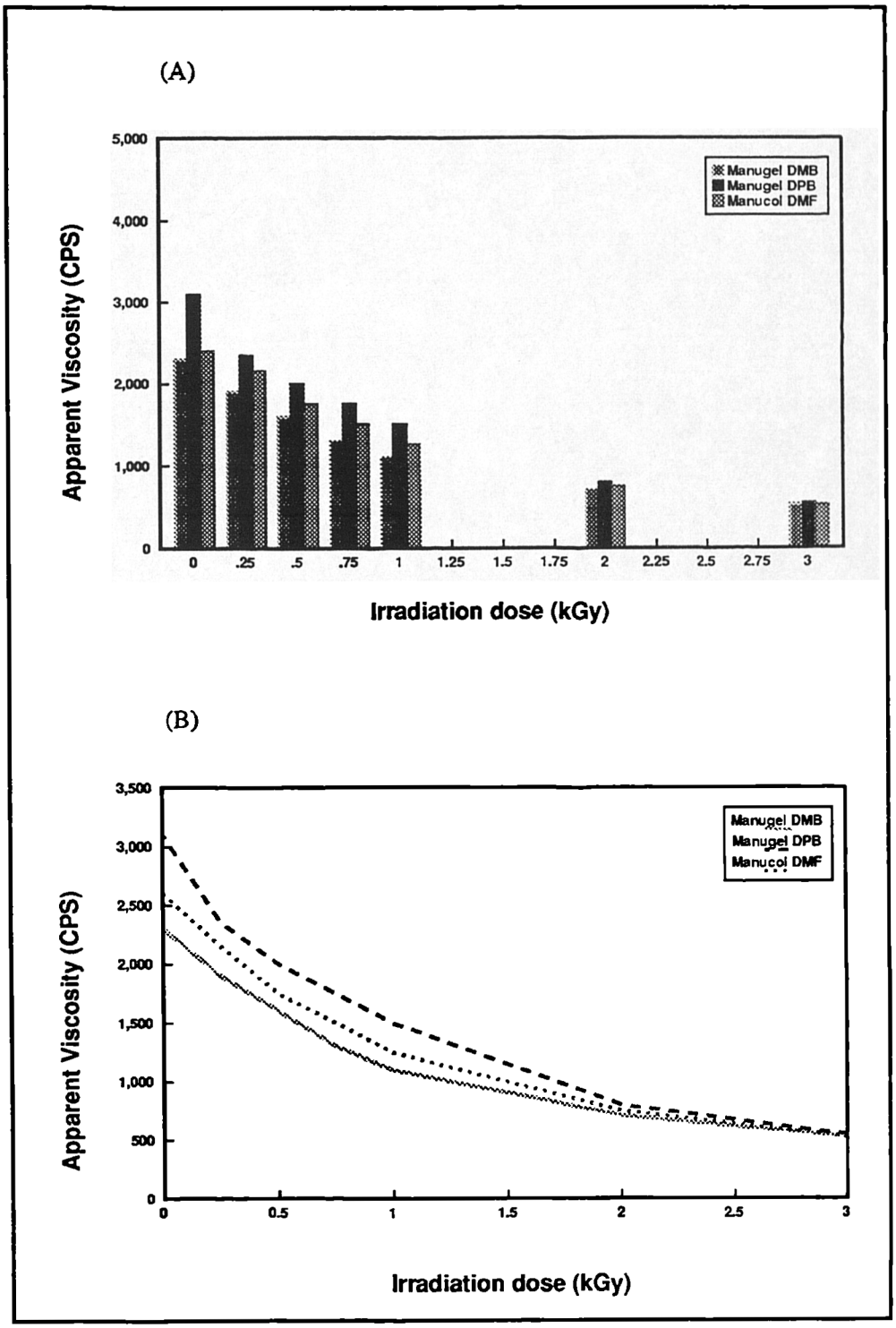


Figure 23: Effect of γ-irradiation in N_2 on the apparent viscosity of three different irradiated sodium alginate solutions (2%), containing mannitol (15%). Using a Brookfield L.V.T. viscometer at 60 rpm and the appropriate spindle. Temperature = 20°C.

(A): As a bar
(B): As a line

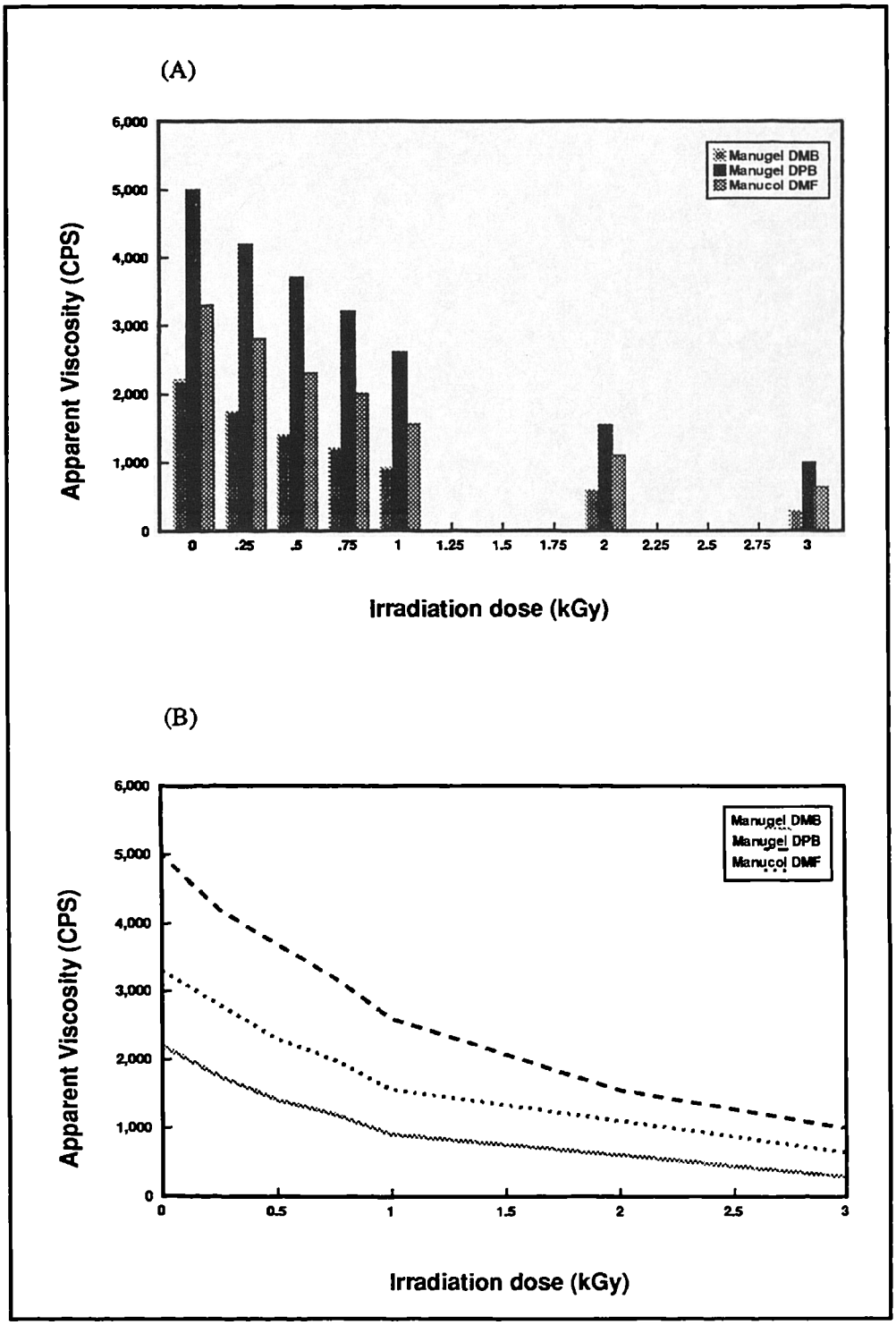


Figure 24: % remaining apparent viscosity for 2% alginate solutions, containing mannitol (15%)irradiated in air and N_2.

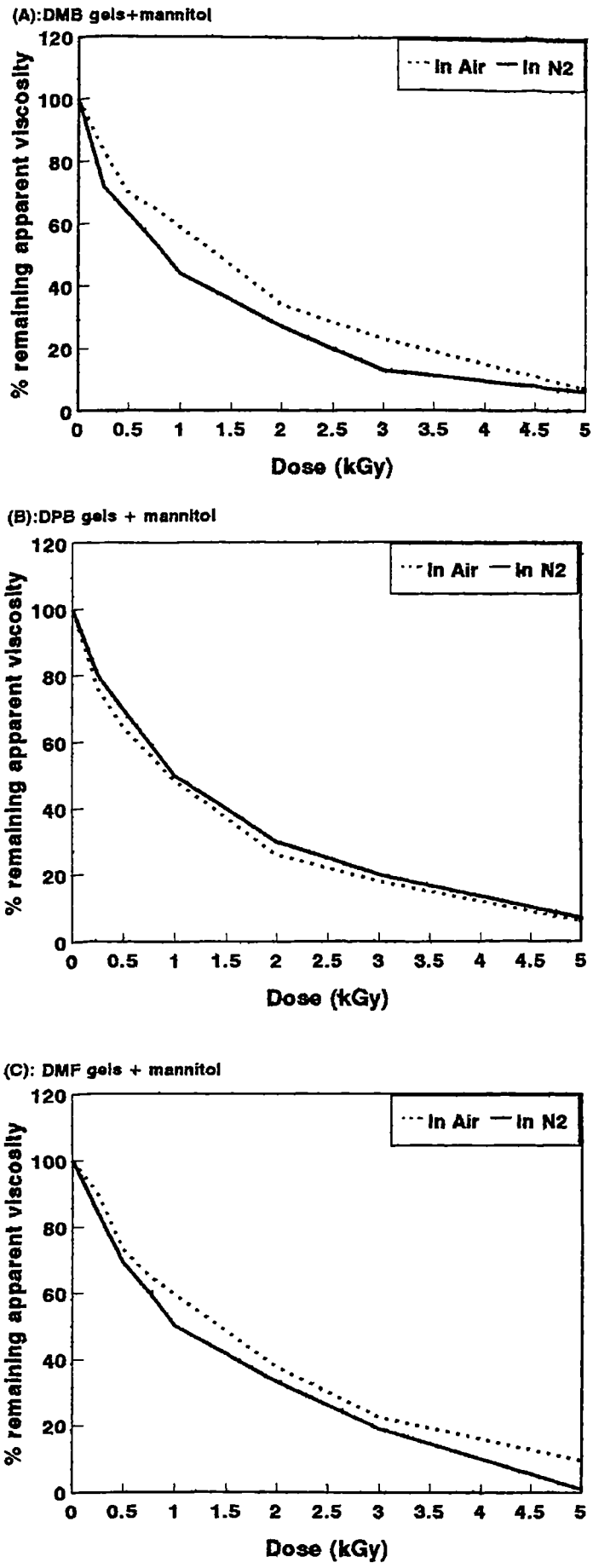


Table XXIV: Apparent viscosity of unirradiated and irradiated manugel DMB gels (2%). Alginate solutions were irradiated to increasing doses then thickened, using three methods described in the text. * = see text

Apparent Viscosity (CPS)

Dose (kGy)	thickening method	N_2 Speed (rpm)			Spindle	Air Speed (rpm)		
		0.6	6	60		0.6	6	60
0	1	>100* 5000 <10* <10	>100 4125 5000 <10	>100 >100 >100 1850	1 2 3 4	2350 <10 <10 <10	>100 2000 3700 <10	>100 >100 1480 1300
	2	>100 >100 <10 <10	>100 >100 11,000 <10	>100 >100 >100 3100	1 2 3 4	>100 37500 29000 <10	>100 >100 7700 <10	>100 >100 1600 1300
	3	←	gel	→	1 2 3 4	←	gel	→
5	1	←	No gel	→	1 2 3 4	←	No gel	→
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	>100 >100 <10 <10	>100 >100 9200 <10	>100 >100 1500 2300	1 2 3 4	>100 >100 <10 <10	>100 >100 6000 <10	>100 >100 1780 <10
25	1	←	No gel	→	1 2 3 4	←	No gel	→
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	←	No gel	→	1 2 3 4	←	No gel	→

Table XXV: Apparent viscosity of unirradiated and irradiated manugel DPB gels (2%). Alginate solutions were irradiated to increasing doses then thickened, using three methods described in the text. * = see text

Apparent Viscosity (CPS)

Dose (kGy)	thickening method	N_2 Speed (rpm) 0.6	N_2 Speed (rpm) 6	N_2 Speed (rpm) 60	Spindle	Air Speed (rpm) 0.6	Air Speed (rpm) 6	Air Speed (rpm) 60
0	1	1250	785	>100	1	1000	920	>100
		<10	875	480	2	<10	775	>100
		<10	<10	680	3	<10	<10	640
		<10	<10	<10	4	<10	<10	<10
	2	>100	>100	>100	1	>100	>100	>100
		35250	>100	>100	2	26000	>100	>100
		28000	6200	1930	3	<10	4300	970
		<10	<10	1350	4	<10	<10	900
	3	←	gel	→	1	←	gel	→
					2			
					3			
					4			
5	1	←	No gel	→	1	←	No gel	→
					2			
					3			
					4			
	2	←	No gel	→	1	←	No gel	→
					2			
					3			
					4			
	3	8450	780	>100	1	>100	>100	>100
		14250	1925	382.5	2	>100	>100	>100
		<10	2100	400	3	<10	8800	1030
		<10	<10	<10	4	<10	<10	<10
25	1	←	No gel	→	1	←	No gel	→
					2			
					3			
					4			
	2	←	No gel	→	1	←	No gel	→
					2			
					3			
					4			
	3	←	No gel	→	1	←	No gel	→
					2			
					3			
					4			

Table XXVI: Apparent viscosity of unirradiated and irradiated manugel DMF gels (2%). Alginate solutions were irradiated to increasing doses then thickened, using three methods described in the text. * = see text

Dose (kGy)	thickening method	N_2 Speed (rpm)			Spindle	Air Speed (rpm)		
		0.6	6	60		0.6	6	60
0	1	>100 >100 11200 14000	>100 >100 >100 3000	>100 >100 >100 1755	1 2 3 4	>100 >100 7000 <10	>100 >100 3200 3500	>100 >100 >100 1355
	2	>100 >100 48000 <10	>100 >100 10000 11000	>100 >100 1900 2300	1 2 3 4	>100 21250 10000 <10	>100 4500 4400 <10	>100 >100 860 1000
	3	←	gel	→	1 2 3 4	←	gel	→
5	1	←	No gel	→	1 2 3 4	←	No gel	→
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	>100 20000 20000 <10	>100 3475 3600 <10	>100 >100 680 <10	1 2 3 4	>100 16000 <10 <10	>100 2200 2400 <10	>100 400 460 <10
25	1	←	No gel	→	1 2 3 4	←	No gel	→
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	←	No gel	→	1 2 3 4	←	No gel	→

thickened using method 3. DMB has viscosity of >100,000, DPB of ~25,000 and DMF ~35,000 cps. It should be mentioned that using this particular equipment, measurement of the viscosity was difficult and the values of viscosity are subject to great margins of error. However it is certain that solutions of irradiated alginates can produce highly viscous solutions using this method (Table XXVII-XXIX).

Method 3 was chosen to thicken (gel) the alginates, after irradiation in the absence and presence of mannitol. Based on the studies previously described, the gelling of sodium alginate is dependent on the concentration of cations and thickening agents present (ie. Ca^{2+}, calcium orthophosphate and gluconolactone respectively).

For manugel DMB (2%) irradiated in air and N_2 to a final dose of 25 kGy no gel was produced and no thickening of the solution occurred. However, when mannitol (20%) was included in the irradiation solution, gelation occurred using half the amount (50%) of the gelling agent used in method 3. The gel, however, was very soft. This experiment was repeated using varying amounts of gelling agent, namely 20%, 40%, 60%, 80% and 100% of the amount of calcium orthophosphate and gluconolactone recommended in the gelation method 3. No thickening or gels formed in alginate solutions irradiated in the presence of mannitol of concentrations of gelling agents less than 50%. In the absence of mannitol, gelation or thickening did not occur at any concentration of gelling agents used.

The gelling agents ie. 20%, 40%, 60%, 80% and 100% were also irradiated individually in air and N_2 to sterilizing doses of the quantity of gelling agent used in method 3, and in a third series of experiments the gelling agents were mixed before irradiation.

Table XXVII: Apparent viscosity of unirradiated and irradiated manugel DMB gels (2%) containing mannitol (15%). Alginate solutions were irradiated to increasing doses then thickened using three methods described in the text.
* = see text

Apparent Viscosity (CPS)

Dose (kGy)	thickening method	N_2 Speed (rpm)			Spindle	Air Speed (rpm)		
		0.6	6	60		0.6	6	60
0	1	>100* >100 >100 >100	>100 >100 >100 12500	>100 >100 >100 3500	1 2 3 4	3750 6000 <10 <10	>100 2500 3800 <10	>100 >100 1550 1350
	2	>100 >100 <10* <10	>100 >100 5000 <10	>100 >100 1400 1000	1 2 3 4	>100 35500 27000 <10	>100 >100 6200 <10	>100 >100 1800 1700
	3	←	gel	→	1 2 3 4	←	gel	→
5	1	<10 <10 <10 <10	<10 <10 <10 <10	71.5 70 <10 <10	1 2 3 4	<10 <10 <10 <!0	585 550 <10 <10	>100 357.5 400 <10
	2	>100 <10 <10 <10	>100 3150 2700 <10	>100 >100 760 <10	1 2 3 4	>100 17500 22000 <10	>100 >100 4200 <10	>100 >100 1230 1500
	3	←	gel	→	1 2 3 4	←	gel	→
25	1	<10 <10 <10 <10	<10 <10 <10 <10	35 <10 <10 <10	1 2 3 4	<10 <10 <10 <10	105 <10 <10 <10	93 85 <10 <10
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	>100 >100 <10 <10	>100 >100 18700 <10	>100 >100 >100 3400	1 2 3 4	>100 >100 117000 120000	>100 >100 >100 22000	>100 >100 >100 7300

Table XXVIII: Apparent viscosity of unirradiated and irradiated manugel DPB gels (2%) containing mannitol (15%). Alginate solutions were irradiated to increasing doses then thickened using three methods described in the text.
* = see text

Dose (kGy)	thickening method	N_2 Speed (rpm) 0.6	N_2 Speed (rpm) 6	N_2 Speed (rpm) 60	Spindle	Air Speed (rpm) 0.6	Air Speed (rpm) 6	Air Speed (rpm) 60
0	1	7150	>100*	>100	1	>100	>100	>100
		9500	3900	>100	2	>100	>100	>100
		23000	5500	>100	3	64000	13000	>100
		<10*	<10	1800	4	<10	<10	2750
	2	>100	>100	>100	1	>100	>100	>100
		41500	>100	>100	2	26500	>100	>100
		31000	8300	>100	3	24000	5800	1500
		<10	<10	1600	4	<10	<10	1350
	3	←	gel	→	1 2 3 4	←	gel	→
5	1	<10	200	>100	1	<10	115	>100
		<10	<10	185	2	<10	<10	100
		<10	<10	180	3	<10	<10	<10
		<10	<10	<10	4	<10	<10	<10
	2	>100	>100	>100	1	>100	>100	>100
		33000	>100	>100	2	18000	4000	>100
		24000	5900	1600	3	<10	3400	1100
		<10	<10	1400	4	<10	<10	900
	3	←	gel	→	1 2 3 4	←	gel	→
25	1	←	No gel	→	1 2 3 4	←	No gel	→
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	>100	>100	>100	1	>100	>100	>100
		19000	2250	>100	2	24500	2875	>100
		2300	2800	600	3	26000	3600	800
		<10	<10	<10	4	<10	<10	<10

Table XXIX: Apparent viscosity of unirradiated and irradiated manucol DMF gels (2%) containing mannitol (15%). Alginate solutions were irradiated to increasing doses then thickened using three methods described in the text.
* = see text

Apparent viscosity (CPS)

Dose (kGy)	thickening method	N_2 Speed (rpm)			Spindle	Air Speed (rpm)		
		0.6	6	60		0.6	6	60
0	1	2500 <10* <10 <10	>100* 1825 2900 <10	>100 >100 1270 1150	1 2 3 4	<10 <10 <10 <10	5350 500 <10 <10	>100 345 380 <10
	2	>100 24500 21000 <10	>100 4400 4900 <10	>100 >100 950 1100	1 2 3 4	>100 20000 <10 <10	>100 4350 4400 <10	>100 >100 830 1400
	3	←	gel	→	1 2 3 4	←	gel	→
5	1	<10 <10 <10 <10	350 <10 <10 <10	>100 2575 270 <10	1 2 3 4	<10 <10 <10 <10	150 <10 <10 <10	>100 125 <10 <10
	2	>100 17500 <10 <10	>100 3000 3200 <10	>100 >100 630 <10	1 2 3 4	>100 12500 <10 <10	>100 1850 <10 <10	>100 377.5 400 <10
	3	←	gel	→	1 2 3 4	←	gel	→
25	1	←	No gel	→	1 2 3 4	←	No gel	→
	2	←	No gel	→	1 2 3 4	←	No gel	→
	3	>100 27000 20000 <10	>100 >100 5800 <10	>100 >100 1270 1500	1 2 3 4	>100 37500 35000 <10	>100 >100 6300 <10	>100 >100 1140 1900

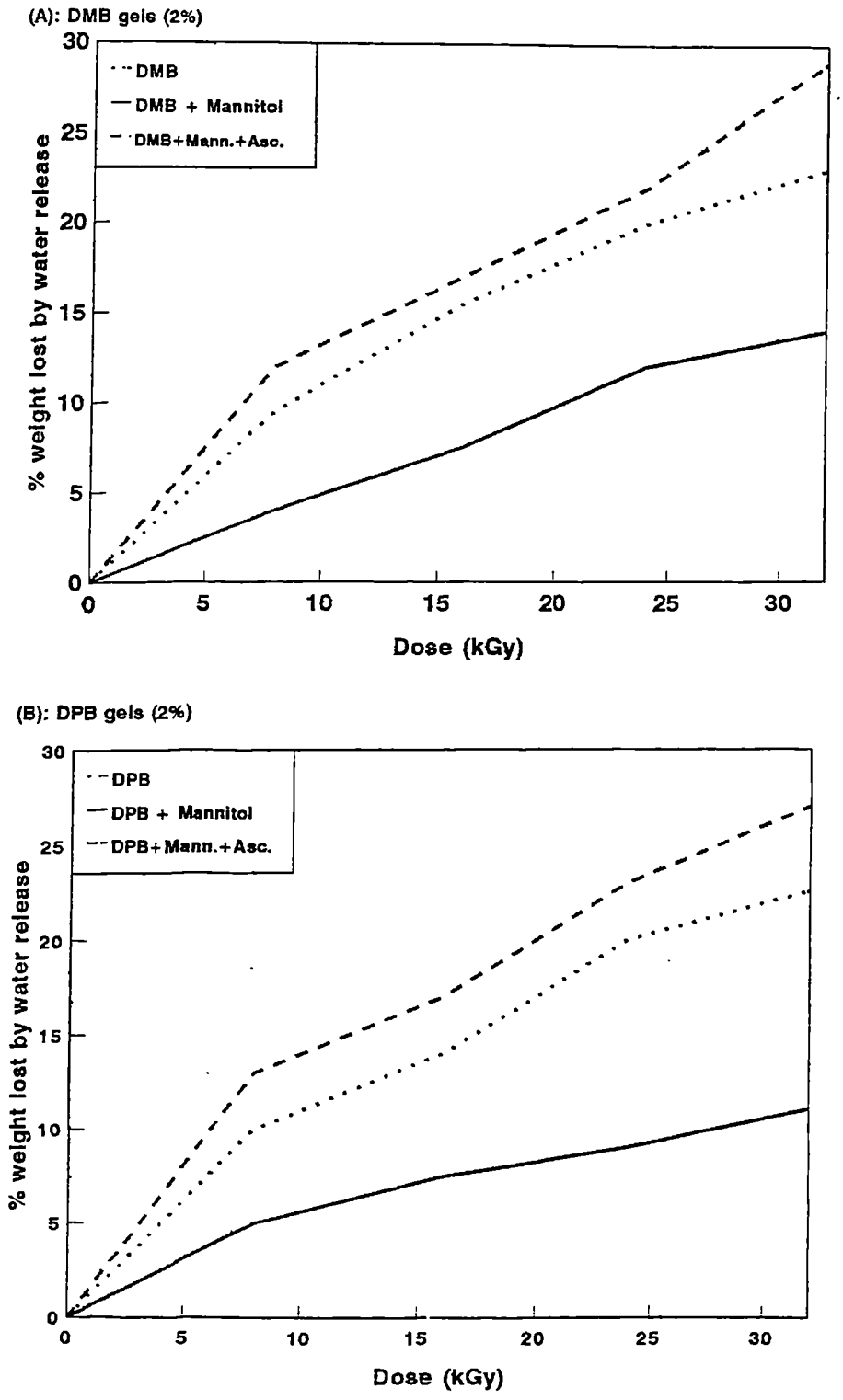
Irradiated mixed gelling agent did not thicken or gel irradiated alginates (with or without mannitol) at any of the concentrations listed above whereas when the irradiated components were added to the alginate-mannitol solution (as in method 3) then gellation and thickening occurred.

From this it can be concluded that sterile components can be used to prepare thickened alginate solutions and gels. In a further series of experiments ascorbic acid (5%) was mixed with manugel DMB (2%) and the gelling agent added as before (using method 3). The solution gelled immediately, water being excluded from the gel. This also happened when mannitol was included in the alginate solution. The alginate appeared to coagulate. The structure of the gel was different from the usual gel, which is more slowly produced over several minutes.

Irradiation of gels causes water release, the amount of which increases up to the sterilizing dose (Figure 25). For 2% DMB gels, approximately 25% of the bound water is released and the gel becomes difficult to handle. When mannitol is included in the gel, the extent of water loss is decreased by an amount of 50%, indicating mannitol is a radiation protector of the gels. However, gels containing mannitol and ascorbate are less stable (more water is released), suggesting that the original gel was not as stable as a gel prepared in the absence of ascorbate (Figure 25A). The data for manugel DPB was very similar to that for DMB, (Figure 25B), again mannitol protects, whereas mannitol/ascorbate decreases gel strength. Irradiated gels prepared using manucol DMF in the absence and presence of mannitol disintegrated at a dose of 16kGy.

An important property of alginate gels in their ability to take up water. The data for

Figure 25: Water release from irradiated manugel DMB & DPB (2%) with/without mannitol (15%) and mannitol and ascorbate (0.5%).



manugel DMB is given in Figure 26. The gels totally immersed in water progressively take up water, such that after one hour the gel has increased by ~30% of its original weight. Gels containing mannitol (15%) took up ~40% of their weight of water and for gels containing mannitol/ascorbate the value was ~20% (Figure 26A).

When the gels were irradiated to doses of 8, 16, 24 and 32 kGy (Figure 26B, C, D and E respectively), those gels containing ascorbate disintegrated after immersion in water in less than one hour. Gels not containing mannitol remain intact, but take up progressively less water at increasing dose, eg. after 8kGy DMB increases by ~22% of its original weight, whereas for 32kGy the value drops to less than 10%. DMB gels containing mannitol increase in weight by >30% of their original weight, after a dose of 8kGy and by ~20% after 32kGy compared with a value of ~30% for an unirradiated DMB gel without mannitol (Figure 26A). The data for water uptake by manugel DPB gels (2%) is given in Figure 27. As for the DMB gel, inclusion of ascorbate decreases the extent of water uptake and inclusion of mannitol increases (slightly) the extent of water uptake. The DPB gel is increased in weight by >100% after one hour. They expand in thickness and diameter and the edges curve upwards (Figure 27A). Irradiated DPB gels (dose 8kGy, Figure 27B) quickly lose their capability to take up water (the DPB-mannitol gel disintegrated after 30 minutes immersion in water at this dose). After 16kGy (Figure 27C) DPB + mannitol + ascorbate retains its integrity and takes up 20% water in one hour but at higher doses it disintegrates in water after ~10 minutes.

Uptake of saline (0.15mol cm^{-3}) by the gels was also studied for DMB gels (Figure 28), the pattern of saline uptake is the same as for water uptake, ie. DMB/mannitol > DMB alone > DMB/mannitol/ascorbate (Figure 28A), though the extent of saline uptake is less than for

Figure 26: Water uptake by unirradiated/irradiated (8, 16, 24 and 32 kGy) manugel DMB (2%) with/without mannitol (15%) and mannitol ascorbate (0.5%).

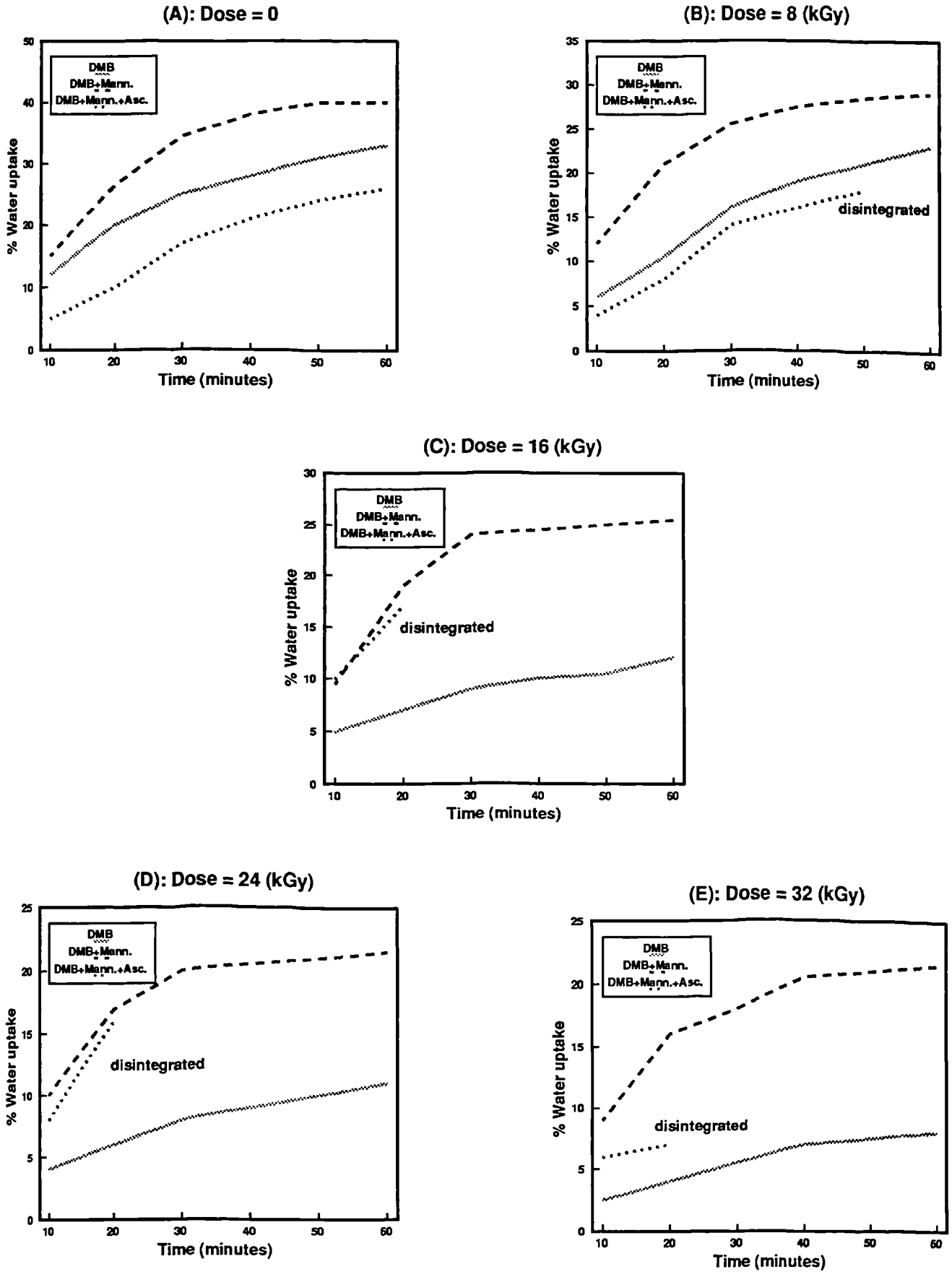
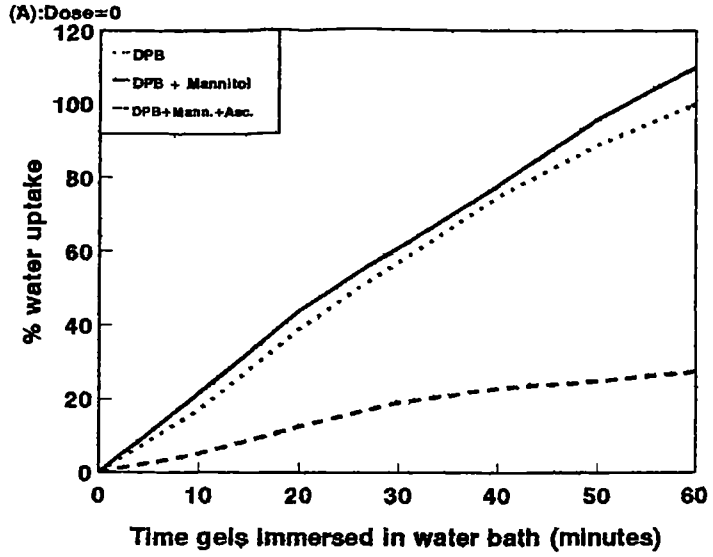
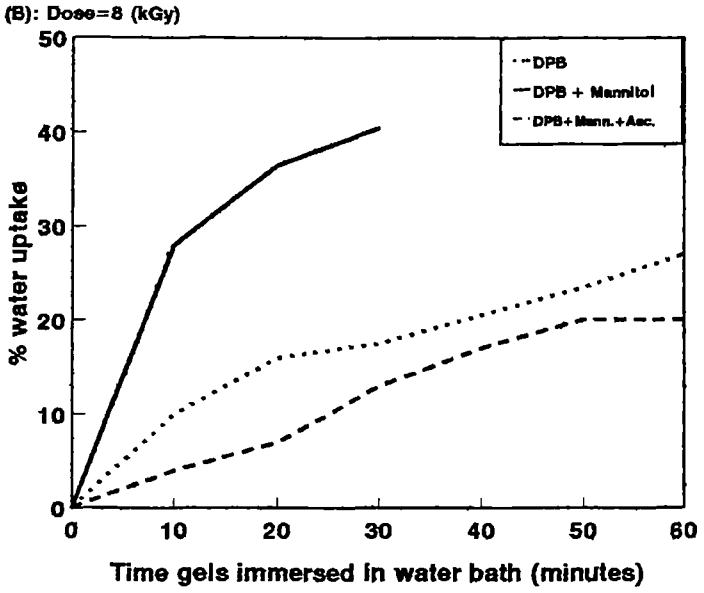


Figure 27: Water uptake by unirradiated/irradiated (8, 16, 24 & 32 kGy), manugel DPB (2%) with/without mannitol (15%) & mannitol ascorbate (0.5%).





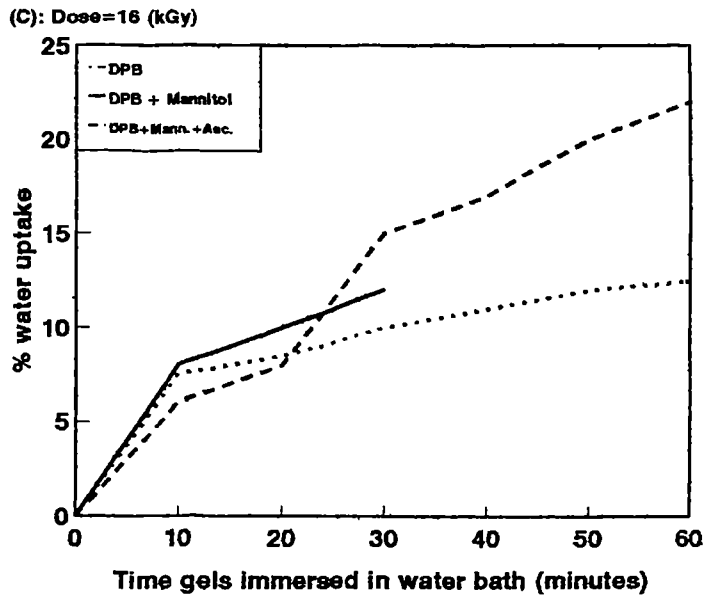
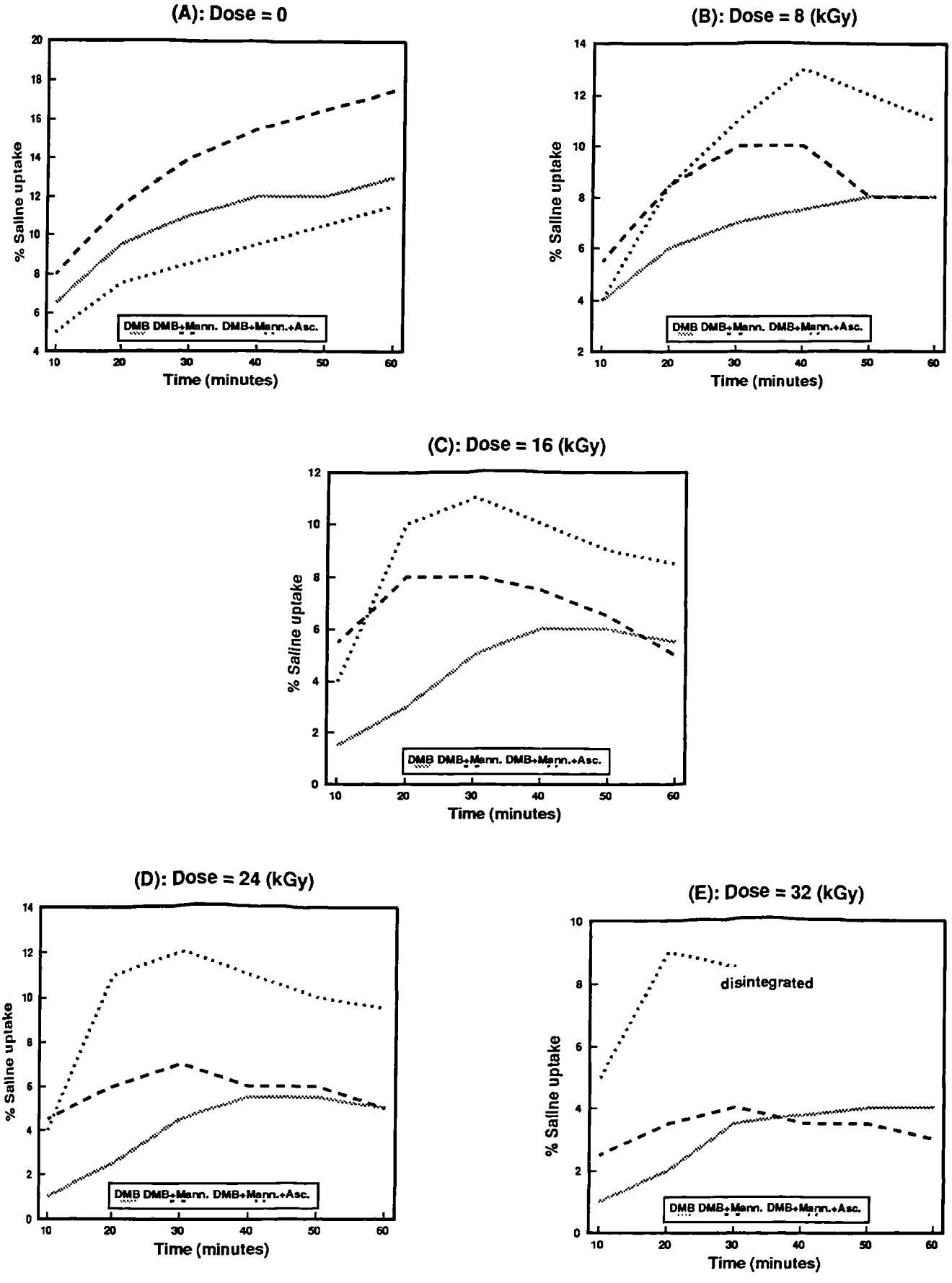


Figure 28: Saline (0.15 mol dm^{-3}) uptake by unirradiated/irradiated (8, 16, 24 and 32 kGy), manugel DMB (2%) with/without mannitol (15%) and mannitol ascorbate (0.5%).



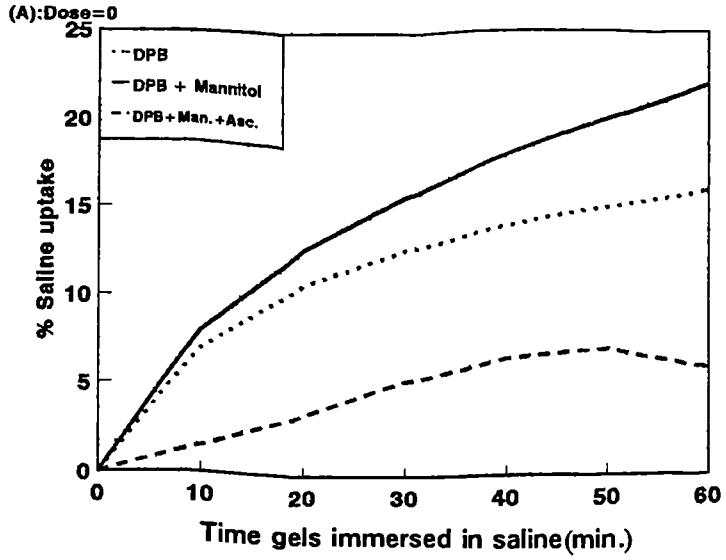
water. This follows the same trend for all the gels studied. The DMB/mannitol/ascorbate gels do not change substantially on irradiation up to 24kGy (Figure 28D). The gels take up ~10-12% saline at each dose employed (Figures 28B, C, D). Irradiated DMB gels take up less saline (a decrease from ~10% to 5% over the range of dose used) and a similar trend is observed for the DMB/mannitol gels. The shapes of the curves in Figures 28, B, C, D are interesting suggesting that saline uptake decreases as the time of exposure to saline increase. Beyond the sterilizing dose (32kGy) curves are similar to those previously discussed, though the DMB/mannitol/ascorbate gel disintegrates after 30mins immersion in saline (Figure 28E). Manugel DPB gels (2%) containing mannitol (15%) take up more saline than both DPB alone and DPB/mannitol/ascorbate gels (Figure 29A). At both 8 and 16kGy the gels have decreased capability to take up saline after an immersion time of ~30-45 minutes for all gels (Figures 29B and C). As for water uptake gels at higher doses disintegrated in saline after ~10 minutes.

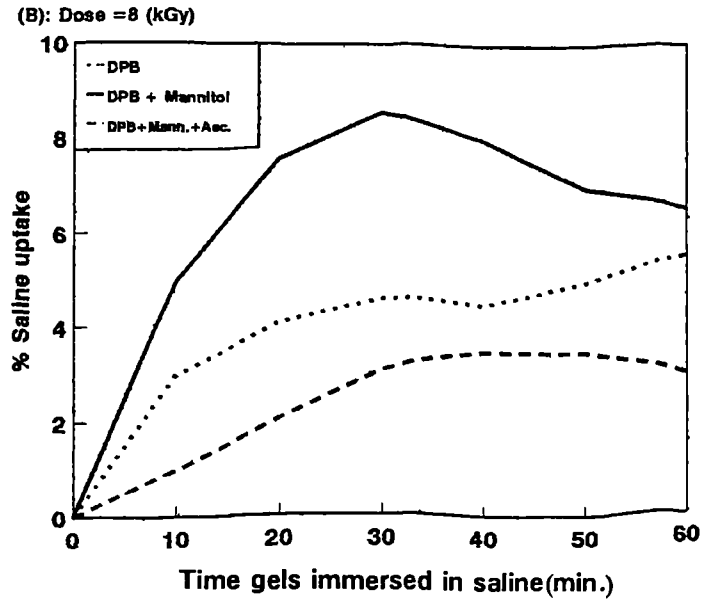
If wet gels are to be used as wound care products they should be capable of bending.

All unirradiated gels can be easily bent through an angle of 180° (Figure 30A and B). Whereas the DMB and DPB/mannitol/ascorbate gels can only bend ~50° after 8kGy, suggesting they are weaker gels. Above 8kGy all gels become less strong and the angle through which they can bend without breaking decreases, eg. ~50° or less after 32 kGy (Figure 30A and B).

Relatively little data has been obtained for manucol DMF gels (2%). They are much weaker than DMB and DPB gels. Unirradiated DMF gels take up water and saline (Figure 31A), but irradiation to only 8kGy causes destruction of the gel structure and DMF alone and

Figure 29: Saline (0.15 mol dm^{-3}) uptake by unirradiated/irradiated (8, 16, 24 and 32 kGy), mariugel DPB (2%) with/without mannitol (15%) and mannitol ascorbate (0.5%).





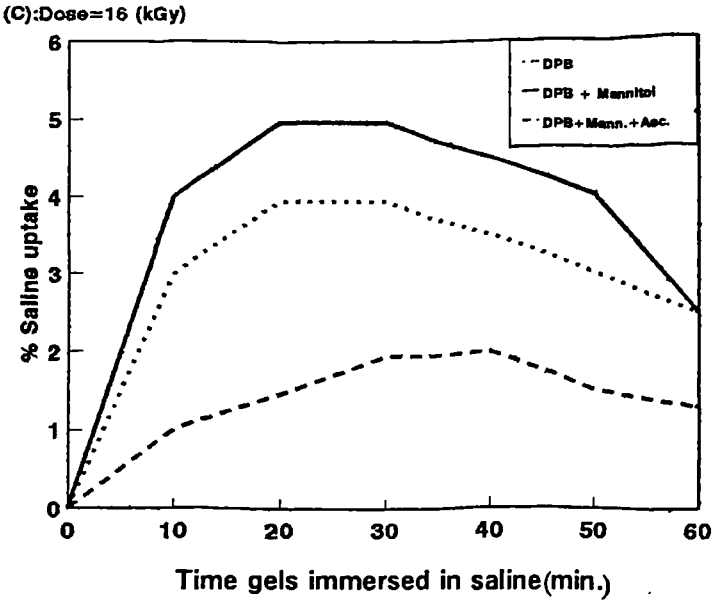
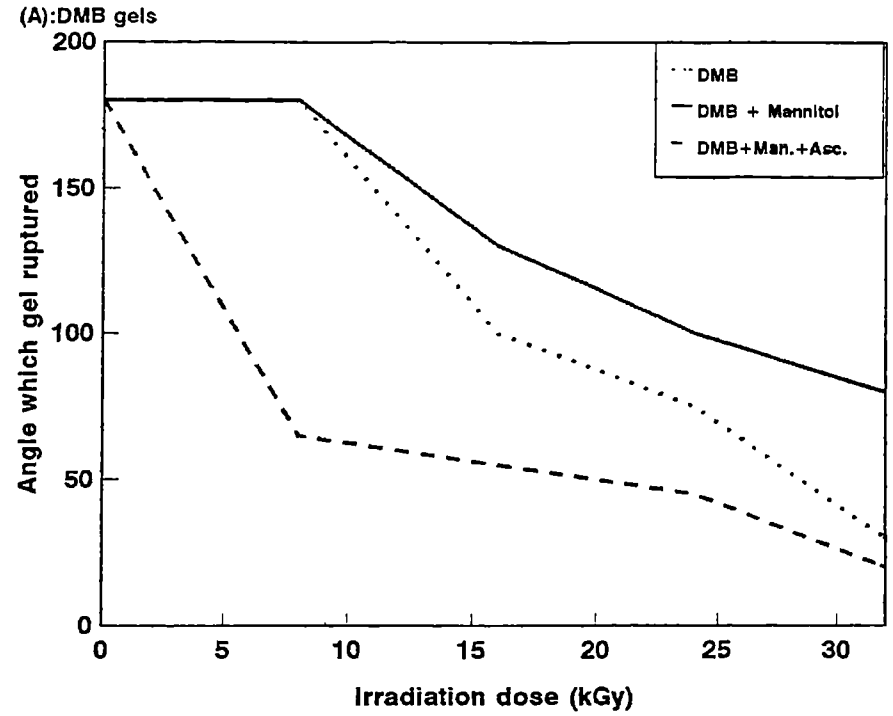


Figure 30: Loss of strength of manugel DMB and DPB gels (2%) with/without mannitol (15%) and mannitol ascorbate (0.5%).



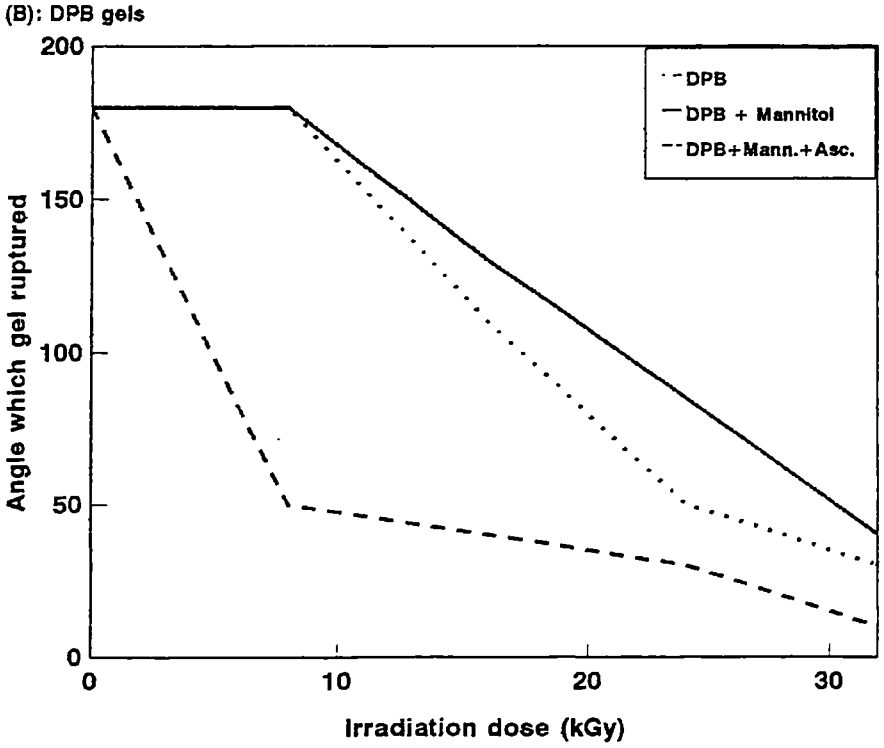
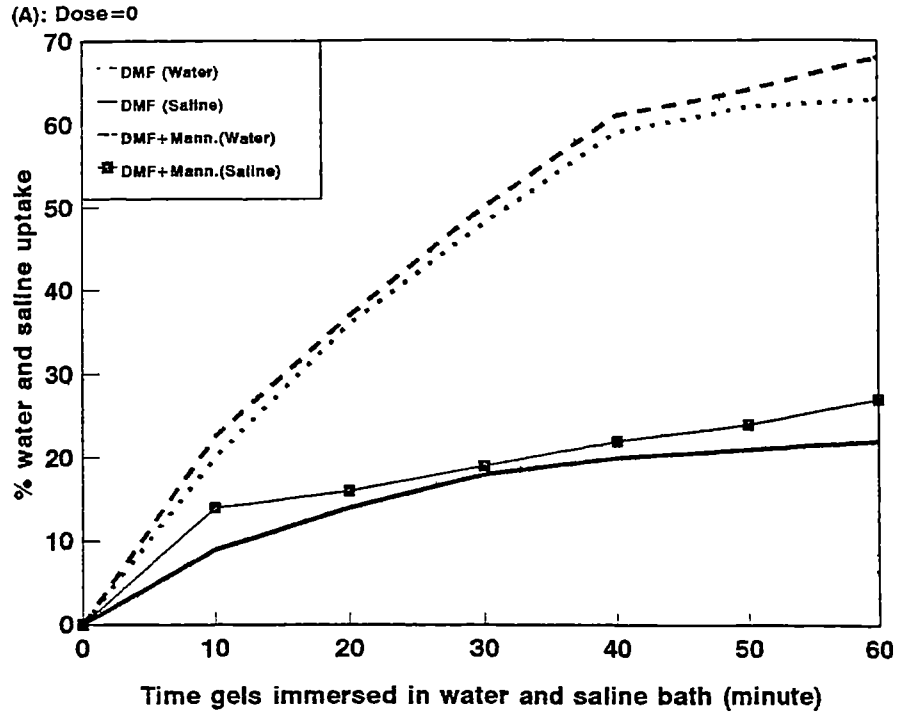
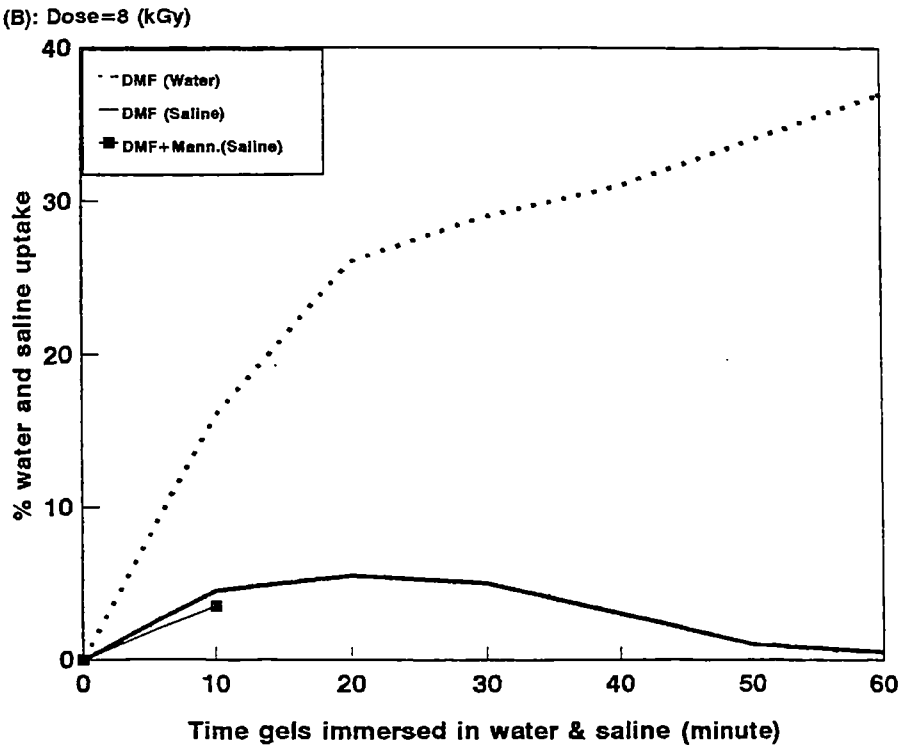


Figure 31: Water and saline (0.15 mol dm^{-3}) uptake of unirradiated/irradiated (8 kGy) manucol DMF gels (2%), with/without mannitol (15%).





DMF/mannitol gels quickly disintegrate in saline (Figure 31B).

Concentrated Gels

DMB gels (2%) were concentrated (by evaporation) to 1/2, 1/3 and 1/4 of their original weights, and water and saline uptake were determined as well as water release and bending capability. In this instance the 2% gels were made so that they were much thicker than those previously used (1cm compared to 3mm), thus enabling them to be concentrated to 5mm, 3.3mm and 2.5mm thickness respectively. Water uptake is much greater for the concentrated gels and their radiation stability increases, the higher is the effective gel concentration (Figure 32A, B, C, D and E). Four and three-fold concentrated gels irradiated to 32kGy behave similarly to unirradiated gels (Figure 32A and 32E).

The data for water uptake with concentrated DMB/mannitol gels are given in figure 33A, B.C.D and E. The shape of the curves indicates an initial weight loss followed by water uptake. All of the concentrated gels appeared to contain precipitated mannitol which on immersion in water washes out from the gel, which may account for the weight loss. Decreased water uptake after 60 minutes immersion, follows irradiation to 32kGy.

It is readily apparent that gel stability increases as gel concentration increases, the gels evaporated to 1/4 of their original weights (ie. 8% DMB gels) take up more water and are more stable after irradiation to a single dose of 32kGy.

Saline uptake was also studied for concentrated DMB gels. Unirradiated gels (x4 concentrated) take up ~40% of their weight of saline, ie. approximately half that of water

Figure 32: Water uptake of unirradiated/irradiated (8, 16, 24 and 32 kGy), manugel DMB gels (2%) as full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 their initial weights.

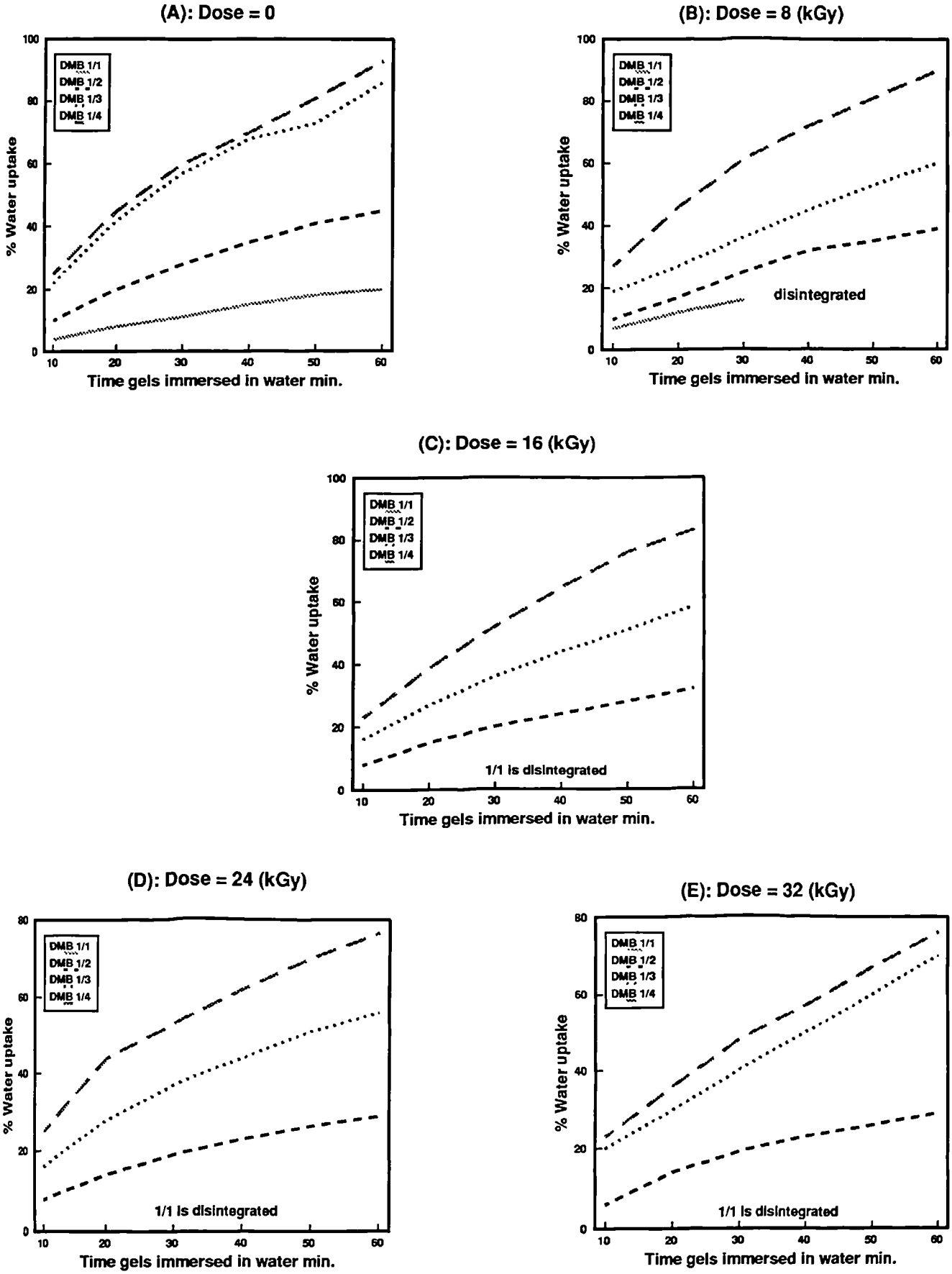
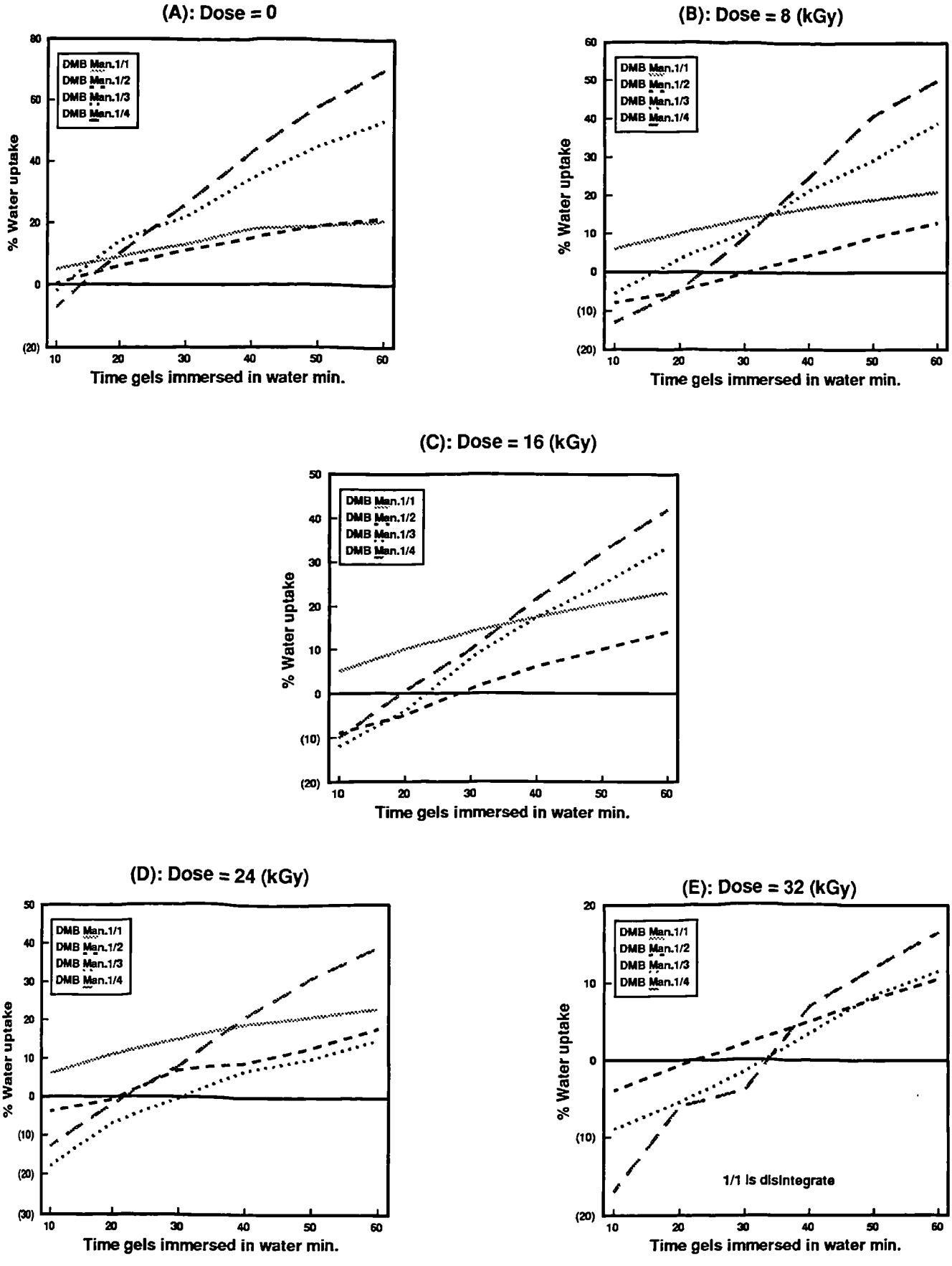


Figure 33: Water uptake of unirradiated/irradiated (8, 16, 24 and 32 kGy), manugel DMB gels (2%) with mannitol (15%) as full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 their initial weights.



uptake (Figure 34A). The amount of saline uptake follows the same pattern as before, namely the higher the gel concentration the greater the amount of saline take up. Irradiated gels (dose 8, 16, 24 and 32kGy) have decreased capacity to absorb saline, though more concentrated gels remain intact after the higher dose used (32kGy), whereas the 2% DMB gel disintegrates after a dose of 24kGy, (Figure 34B, C, D and E).

Saline uptake was measured for unirradiated DMB (2%)/mannitol (15%) gels, that had been concentrated by a further of 2, 3.33 and 4. At these concentrations mannitol precipitates, the gel being covered with the white powder. Immersed in saline the gels initially appear to lose weight, which is presumed to be due to the mannitol precipitate being washed out of the gel. After ~10 minutes the gels all take up saline in the usual way, the most concentrated increased in weight by almost 30% (Figure 35A).

The highest concentrated gels lose ~15-20% of their initial weight after irradiation and immersion in saline (due to loss of mannitol) (Figure 35, B, C, D and E) and the gel irradiated to 8kGy (Figure 35B) regains ~10% of its new weight due to saline uptake. At higher doses, saline uptake by gels after this initial washing out of the mannitol decreases compared with the unirradiated gels (Figure 35A).

Water release by irradiated concentrated DMB (2%) gels, ie. 8, 6 and 4% gels is markedly less than for the full gel (2%), (Figure 36). Furthermore, the concentrated gels, containing mannitol showed little or no release of water, though they again approved to be covered in a white powder presumed to be precipitates mannitol (Table XXX).

Figure 34: Saline (0.15 mol dm^{-3}) uptake by unirradiated/irradiated (8, 16, 24 and 32 kGy), manugel DMB gels (2%) as a full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 of their initial weights.

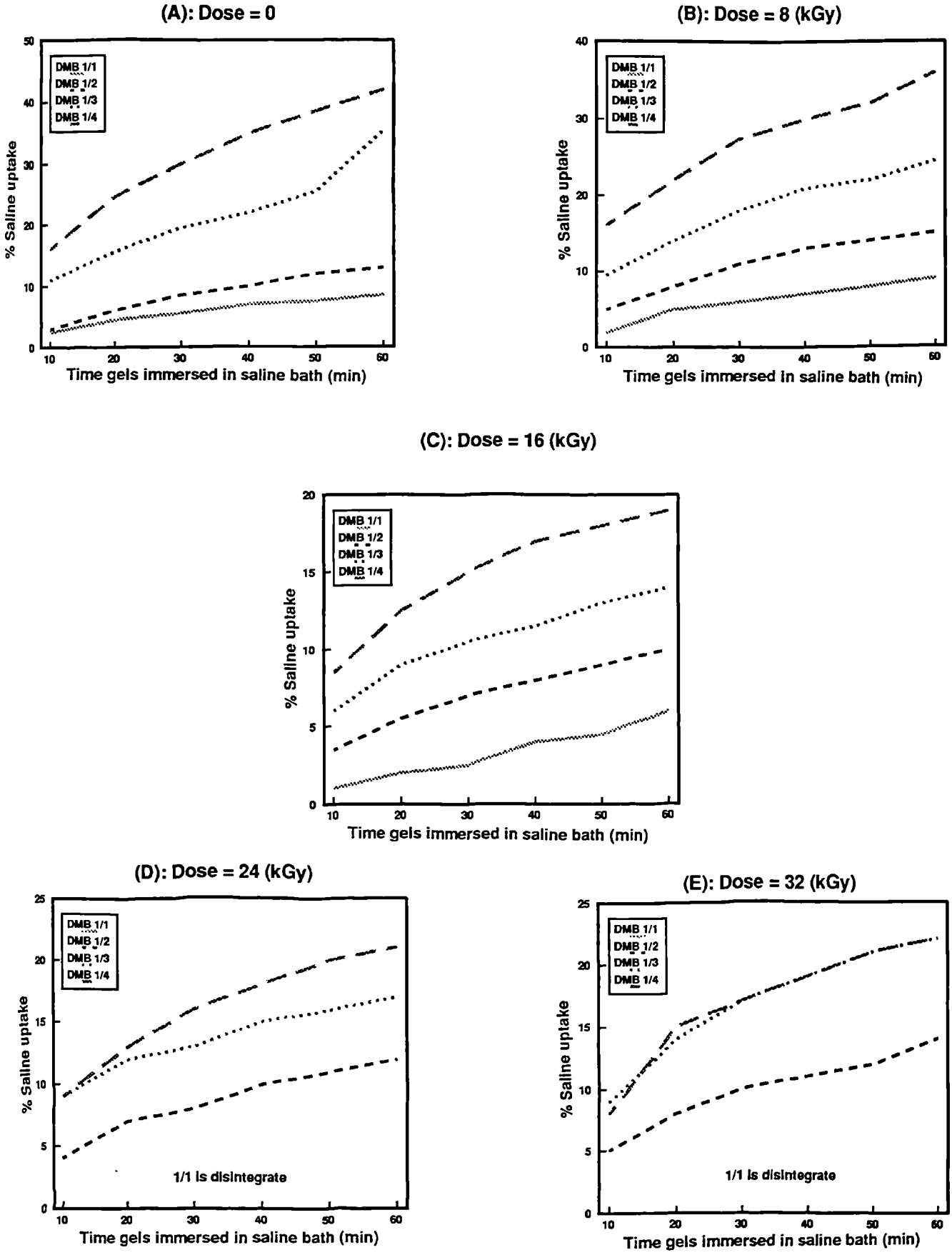


Figure 35: Saline (0.15 mol dm^{-3}) uptake by unirradiated/irradiated (8, 16, 24 and 32 kGy), manugel DMB gels (2%) with mannitol (15%) as a full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 of their initial weights.

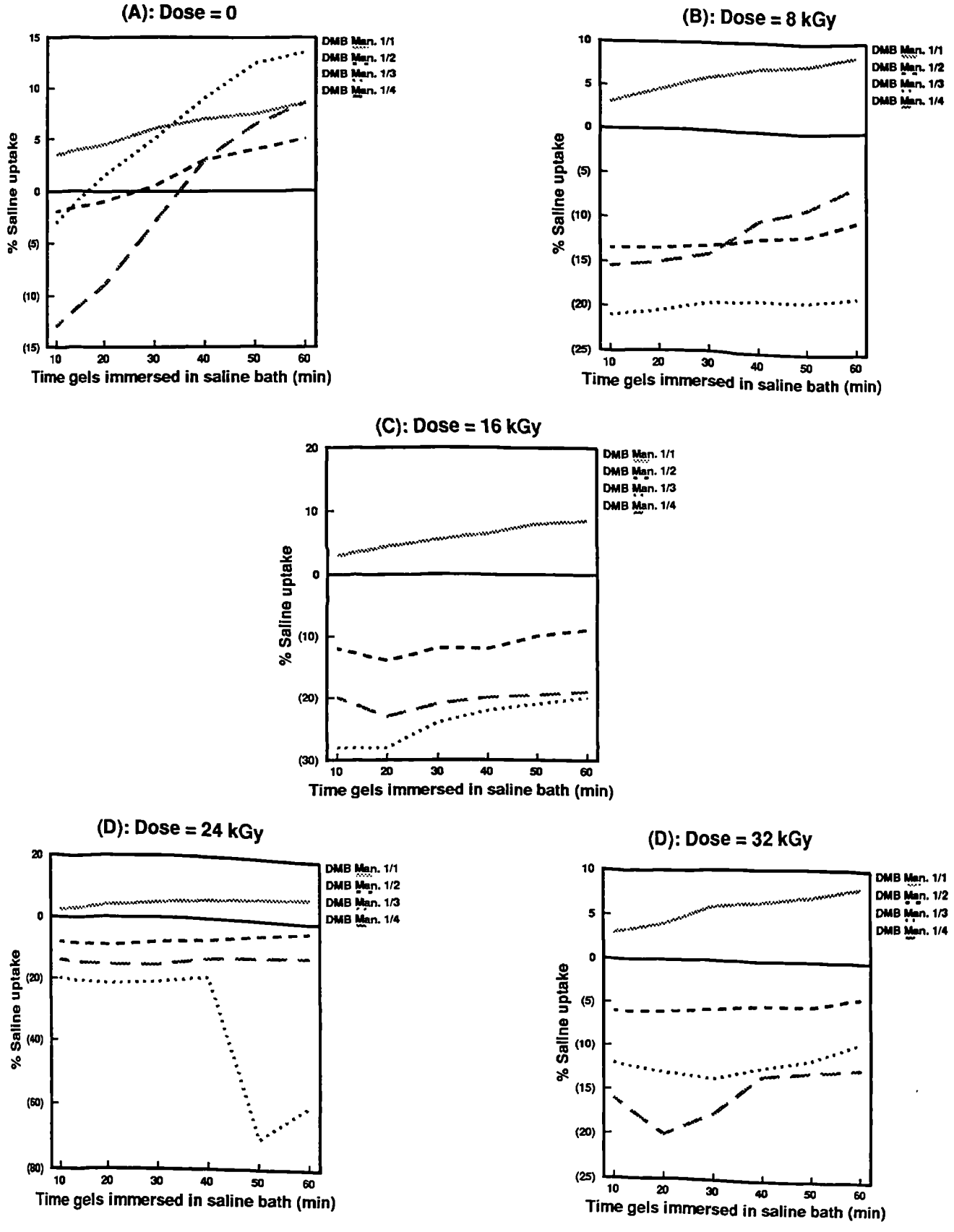


Figure 36: Water Release by irradiated manugel DMB (2%) as full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 of their initial weights.

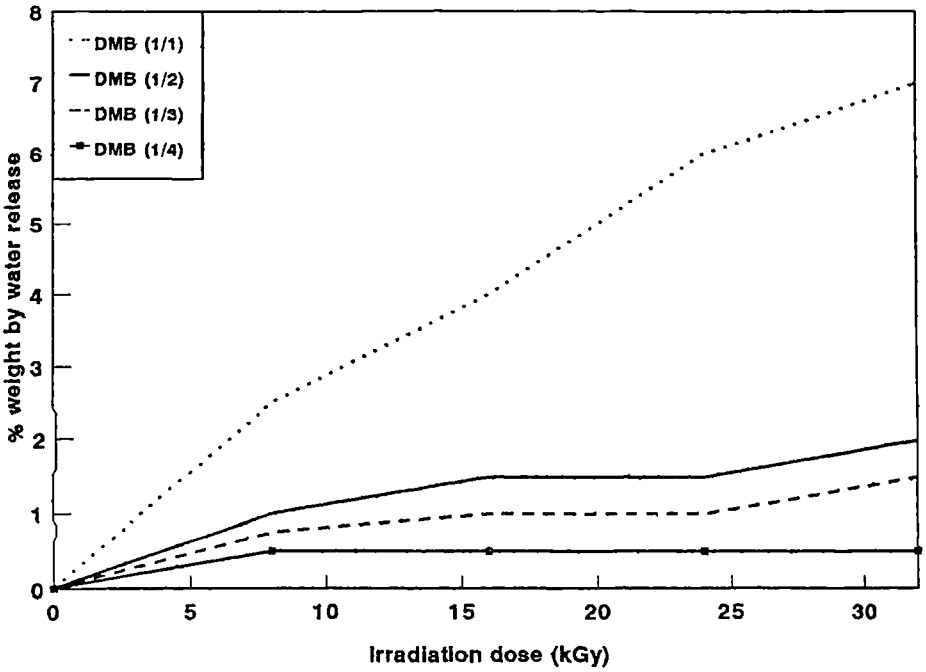


Table XXX: Water release by irradiated manugel DMB (2%) with mannitol (15%) as full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 of their initial weights.

Sample	% Weight water release			
	Dose (kGy)			
	8	16	24	32
1/1	1.66	2.5	2.5	2.83
1/2	0.16	0	0	0
1/3	0	0	0	0
1/4	0	0	0	0

The dry irradiated gels do, however, break on bending above a dose of 8kGy though the higher the effective gel concentration the greater is the angle through which bending can occur before rupture (Figure 37A).

Gels were immersed in water for one hour and then tested for their bending capabilities. Increased radiation dose causes loss of gel strength, all gels can bend only ~50-80° after a dose of ~32kGy (Figure 37B).

The data for DMB/mannitol gels is given in Figure 37C and D. Irradiated gels break on bending though for irradiated gels immersed in water the four-fold concentrated gels appear to be completely stable even up to a dose of 32kGy (Figure 37D).

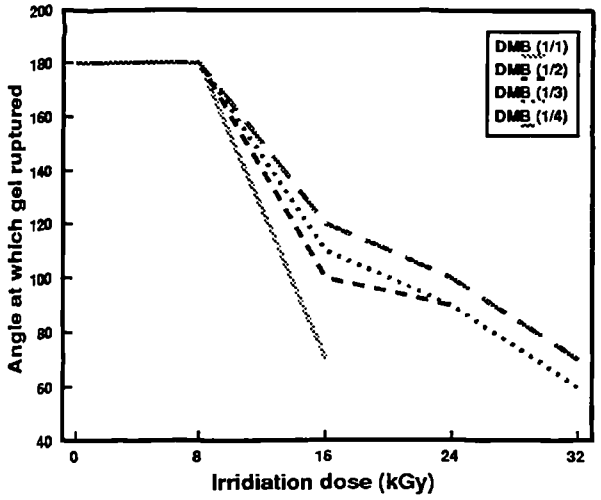
In a further series of experiments, the effect of immersion of the concentrated DMB gels in saline for extended periods of time was investigated. The 2% gels are intact after 24 hours although disintegrated after 72 hours. The concentrated gels (4%, 6% and 8%) are all intact after 72 hours (Figure 38A). Gels were irradiated to 10, 20 and 30kGy (Figure 38B, C and D). 2% gels disintegrate after 20kGy after 60 minutes immersion, 4% and 6% gels irradiated to 30kGy develop cracks, whereas the 8% gel remains intact (Figure 38D) up to 24 hours immersion in saline.

The effect of varying (a) the concentration of mannitol in gel and (b) the amount of mannitol/ascorbate in the gel was also studied.

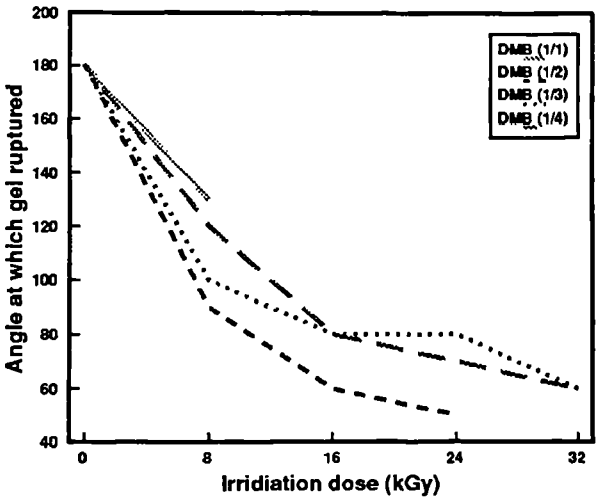
2% DMB/20% mannitol with or without ascorbate (0.5%) readily take up water up to 24 hours (Figure 39A). The amount of saline uptake by these gels is considerably reduced and

Figure 37: Loss of strength and bending ability by manugel DMB gels (2%) with/without mannitol (15%) as a full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 of their initial weights (A and C) and after 60 minutes of water uptake (B and D).

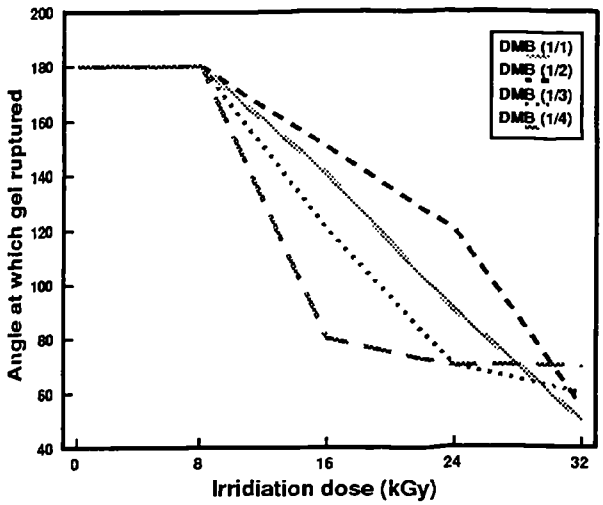
(A): As DMB gels (2%) and dried up to 1/2, 1/3 and 1/4



(B): As (A), after 60 mins water uptake



(C): DMB gel (2%) with mannitol (15%)



(D): As (C) after 60 mins. water uptake

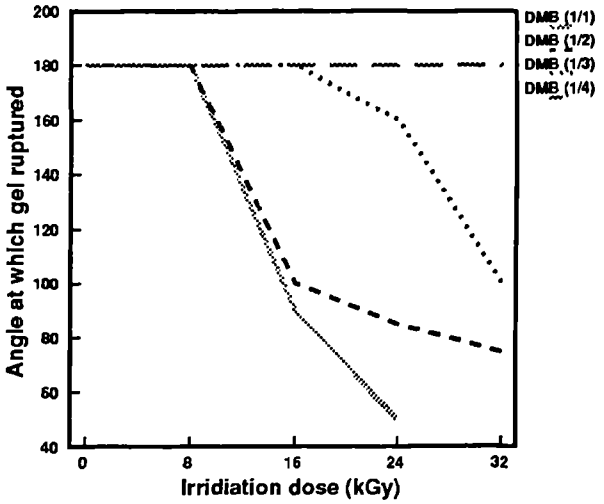
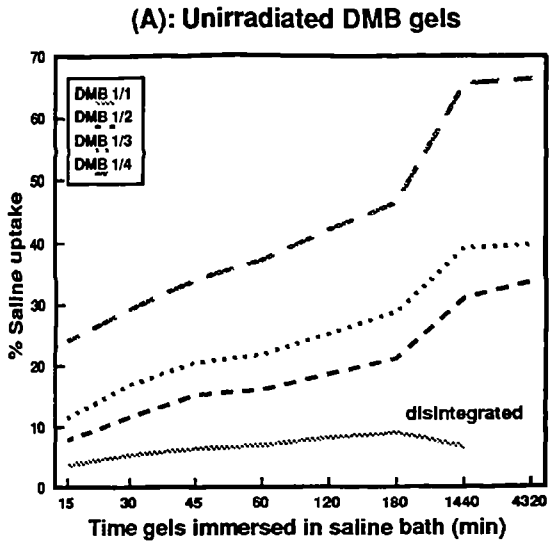
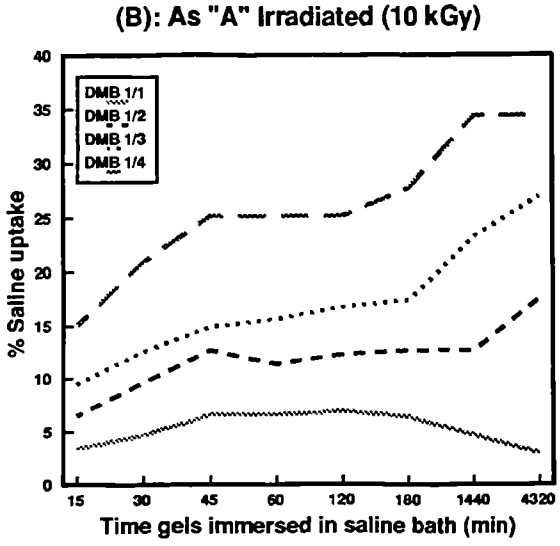
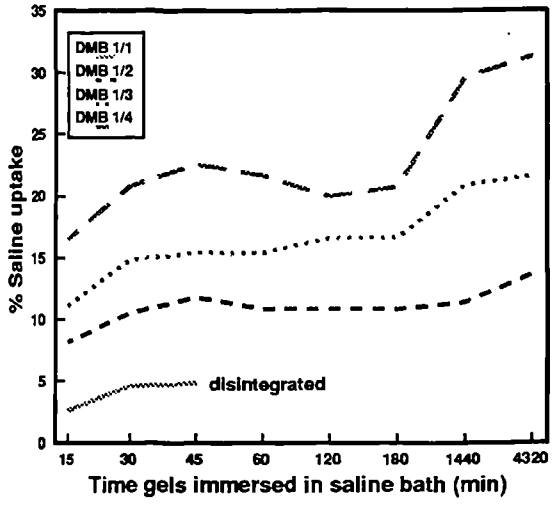


Figure 38: Saline (0.15 mol dm^{-3}) uptake by unirradiated/irradiated (10, 20 and 30 kGy), manugel DMB gels (2%) as a full gel (1/1) and gels dried to 1/2, 1/3 and 1/4 of their initial weights.







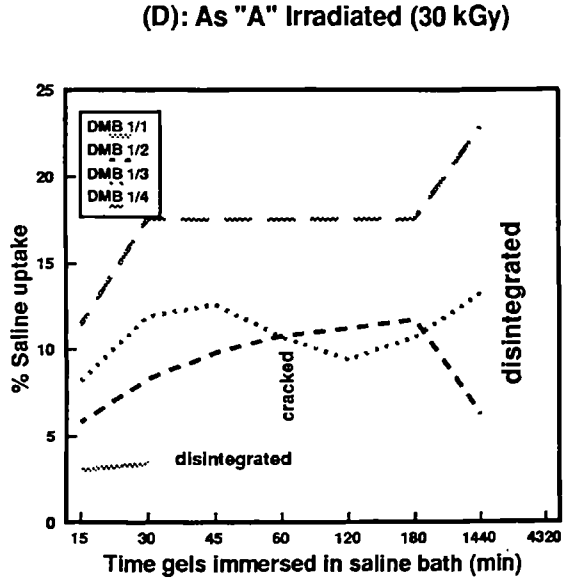
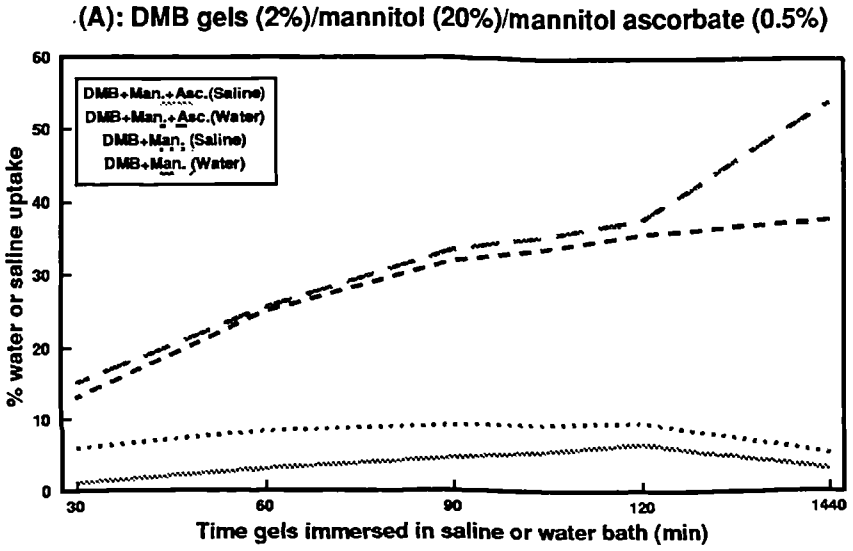
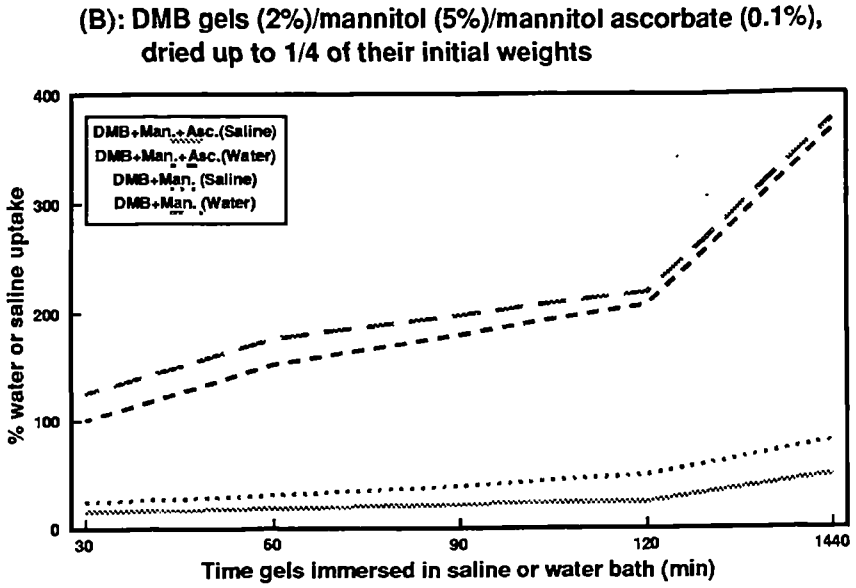
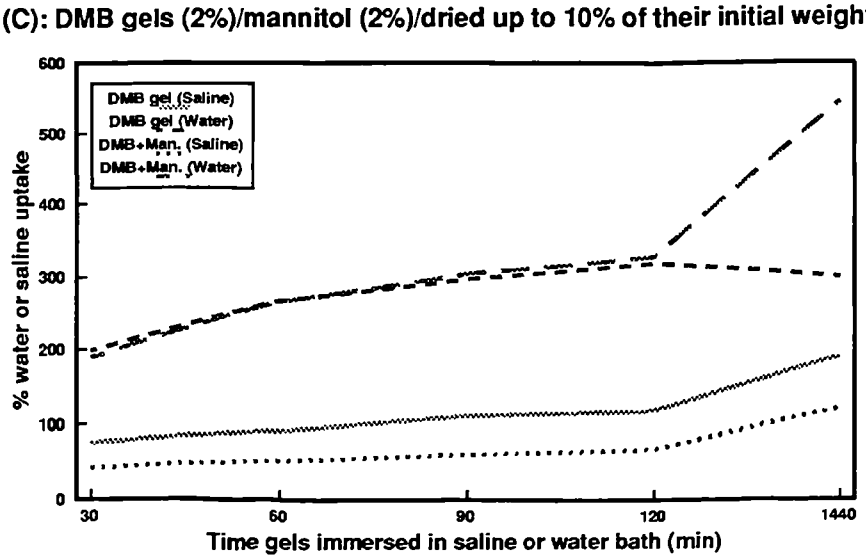


Figure 39: Saline (0.15 mol dm^{-3}), or water uptake by unirradiated manugel DMB gels (2%) with/without mannitol and with/without ascorbate.







after 24 hours the saline uptake has decreased, suggesting loss of gel properties.

2% DMB/5% mannitol with or without ascorbate (0.1%) dried to 1/4 of its original weight (ie. 4 times concentrated), take up 400% of its original weight in water and by 50% in saline (Figure 39B).

2% DMB gels with or without mannitol (2%) concentrated by a factor of 10 take up ~500% of the original weight in water and >100% of their weight in saline (Figure 39C).

The data for the corresponding gels irradiated to 30kGy are given in table XXXI. Now the gels (A in table XXXI) are unstable and mannitol/ascorbate gels disintegrated in saline after 30 minutes, whereas in the absence of ascorbate the gels lose weight up to 2 hours immersion in saline, then crack.

Gels four fold concentrated (B in table XXXI) remain intact in saline, though in water they disintegrated after 30 minutes. Gels 10 fold concentrated (C in table XXXI) which contain 20% alginate and 20% mannitol are stable in saline and also take up a lot of water within 30 minutes but in the latter case they become "mushy" and non-useable.

<u>Xanthan Gum:</u>

An in depth rheological characterization of gum solutions requires the determination of its viscosity as a function of varying shear rate. A concentric cylinder with suitable spindles covering a wide range of shear rates is necessary. When viscosity-shear rate curves were required over a range of shear rates, it was sometimes necessary to use more than three different spindles to cover low, intermediate and high shear rate ranges. Shear rate overlap

Table XXXI: Saline (0.15mol dm^{-3}) and water uptake by irradiated (30kGy) manugel DMB (2%) gel containing mannitol (20%) and mannitol ascorbate (0.5%) as full gel (100%), gel containing mannitol (5%) and mannitol ascorbate (0.1%) as dried gel (25%) and gel with/without mannitol (2%) dried up to 10% of their initial weights.

Sample	% weight measurement by uptake									
	Time: Saline					Time: Water				
	30	60	90	120	1440	30	60	90	120	1440
(A)										
Alg. (2%) + mannitol (20%) + Ascorbate (0.5%) (100% gel)	4.40	-	-	-	-	-	-	-	-	-
Alg. (2%) + mannitol (20%) (100% gel)	-1.68	0.84	-0.28	-1.68	cracked	-	-	-	-	-
(B)										
Alg. + mannitol (5%) + Ascorbate (0.1%) (25% gel)	7.69	7.69	7.69	6.59	9.89	28.57	-	-	-	-
Alg. + mannitol (5%) (25% gel)	12.37	10.30	10.30	11.34	13.40	32.98	-	-	-	-
(C)										
Alg. (2%) (10% gel)	24.52	28.3	33.96	33.96	41.5	150	-	-	-	-
Alg. + mannitol (2%) (10% gel)	38.63	40.9	45.45	47.72	63.63	115.27	-	-	-	-

between data ensures the integrity of the data, since a systematic error due to a particular measurement will be detected.

Data for viscosity measurements was therefore taken over a wide range of shear-rates for each spindle used. Figure 40 illustrates the shear rate overlap between the different spindles used, at a minimum and maximum spindle speed of 0.3 and 60rpm respectively.

Figure 41 presents typical rheology of xanthan gum solutions, plots of shear rate vs. shear stress (A), apparent viscosity vs. shear stress (B) and apparent viscosity vs. shear rate (C). The shear rate data is given as rpm (A and B) and sec^{-1} (C) respectively. Figure 42 shows the effect of addition of NaCl on the apparent viscosities of xanthan gum (1%) and in combination with locust bean gum (LBG) 1%. The apparent viscosity of the gums containing NaCl is greater than that in the absence of NaCl. There is a similar decrease in apparent viscosity with increasing shear rate (sec^{-1}), indicating that xanthan gum and locust bean gum *are pseudoplastic, similar to that found for alginate solutions.*

Addition of mannitol (20%) to xanthan gum solutions (1%) and mannitol/ascorbic acid (10^{-2} mol dm^{-3}) results in a small increase in apparent viscosity (Figure 43). Locust bean gum (1%) was also included in the above solutions (Figure 44). The results were similar to the data in figure 43 except that the viscosities were all substantially increased and the decrease in viscosity with increased shear rate again is clear evidence of the pseudoplastic nature of these solutions. Figure 45 shows the apparent viscosity of separate solutions of xanthan gum (1%) and locust bean gum (LBG) 1% and of their solutions in the presence of 20% mannitol and with mannitol and ascorbic acid (10^{-2} mol dm^{-3}). All solutions have varying degrees of pseudoplasticity, xanthan gum/mannitol/ascorbic acid being the most pseudoplastic, and

Figure 40: Schematic representation of the changes occurring in shear rate using (overlap) between different spindle numbers (16,18,25,31 and 34) at a minimum spindle speed of 0.3 rpm and maximum spindle speed of 60rpm. Measurements were recorded using 8 speeds, namely 0.3, 0.6, 1.5, 3, 6, 12, 30 and 60 rpm, spindle speed is related to shear rate as in tables, XIII and XIV.

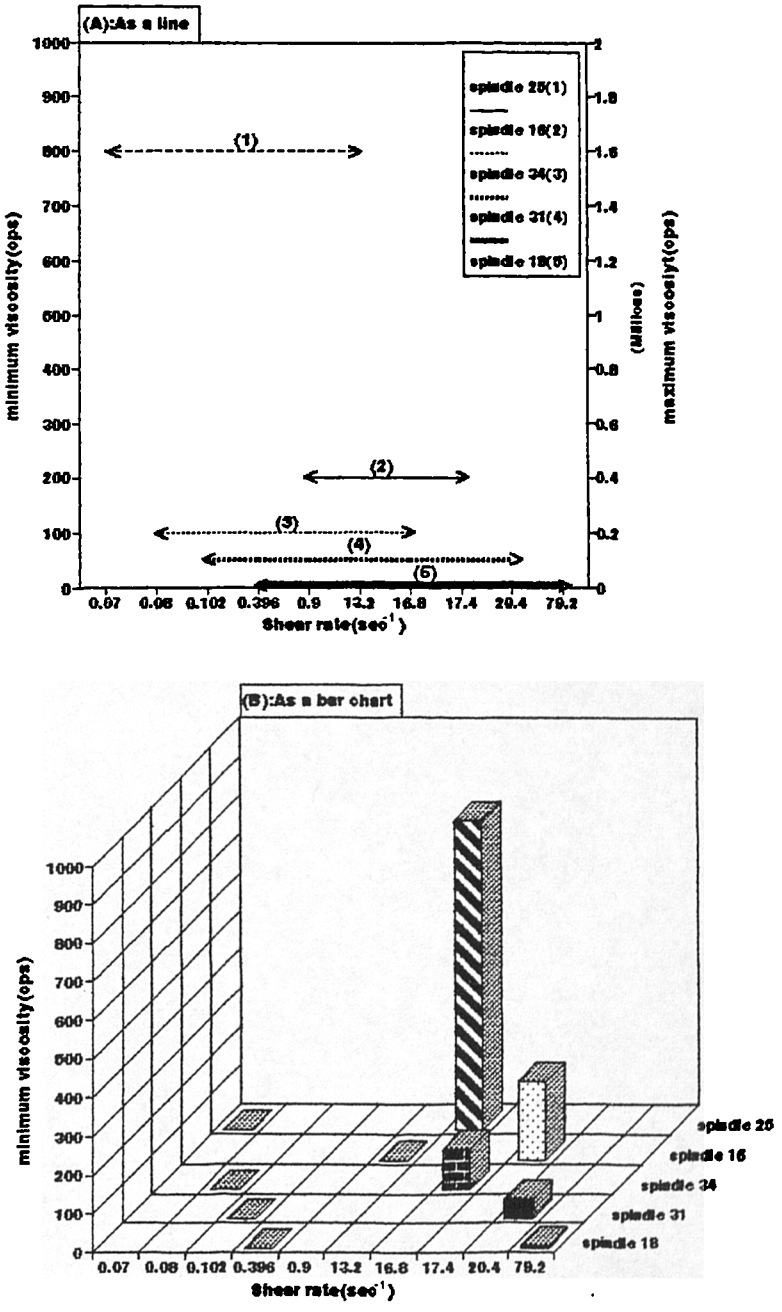


Figure 41: Rheology of xanthan gum solutions (1%), plots of shear rate vs shear stress (A), apparent viscosity vs shear stress (B) and apparent viscosity vs shear rate (C). The data was obtained using a Brookfield L.V.T. viscometer, spindle number 31. Temperature= 20°.

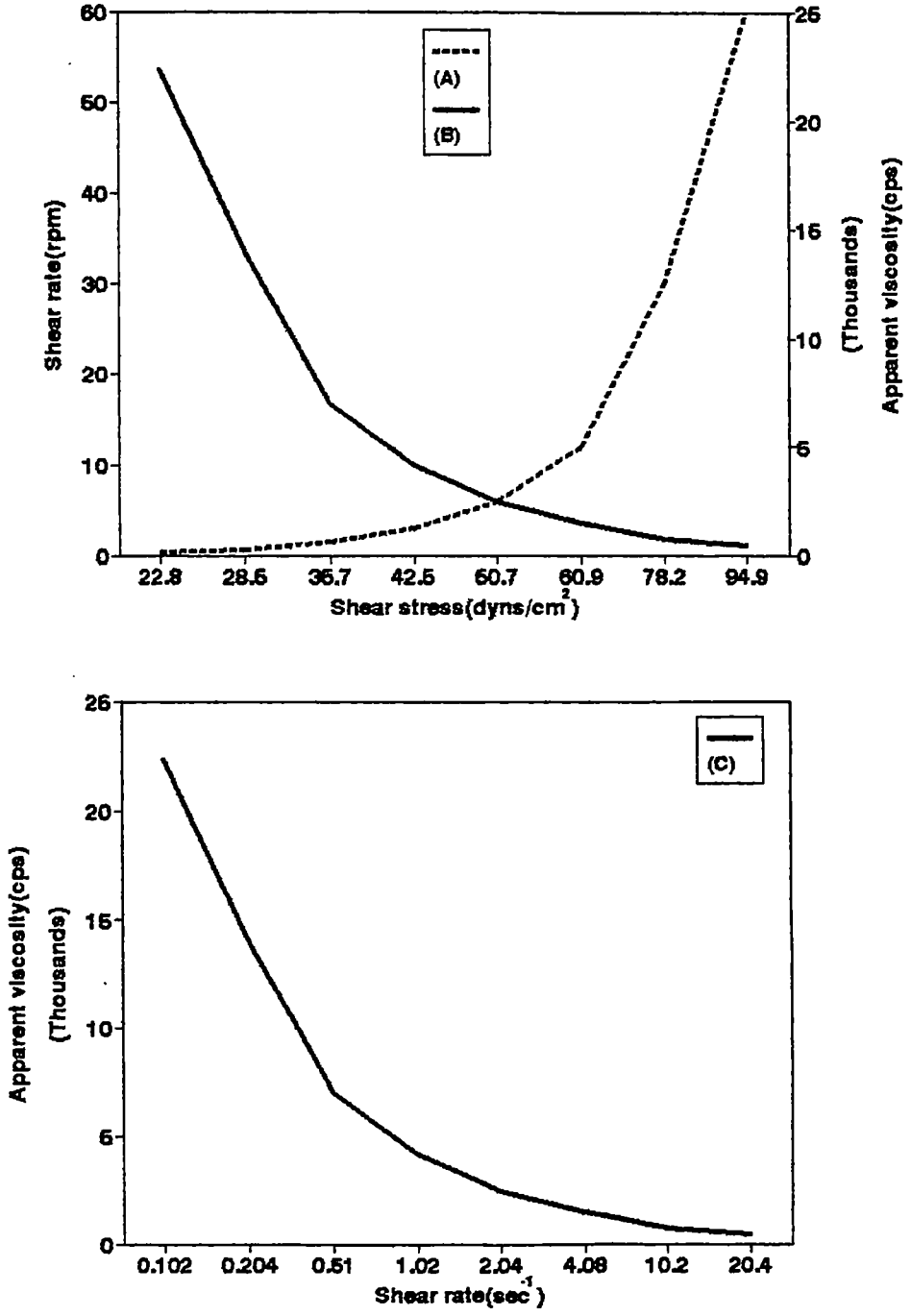


Figure 42: Measurement of apparent viscosity as a function of shear rate for air saturated xanthan gum (1%), xanthan gum (1%) + LBG (1%), xanthan gum (1%) +NaCl (0.1%) and xanthan gum (1%), LBG (1%) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

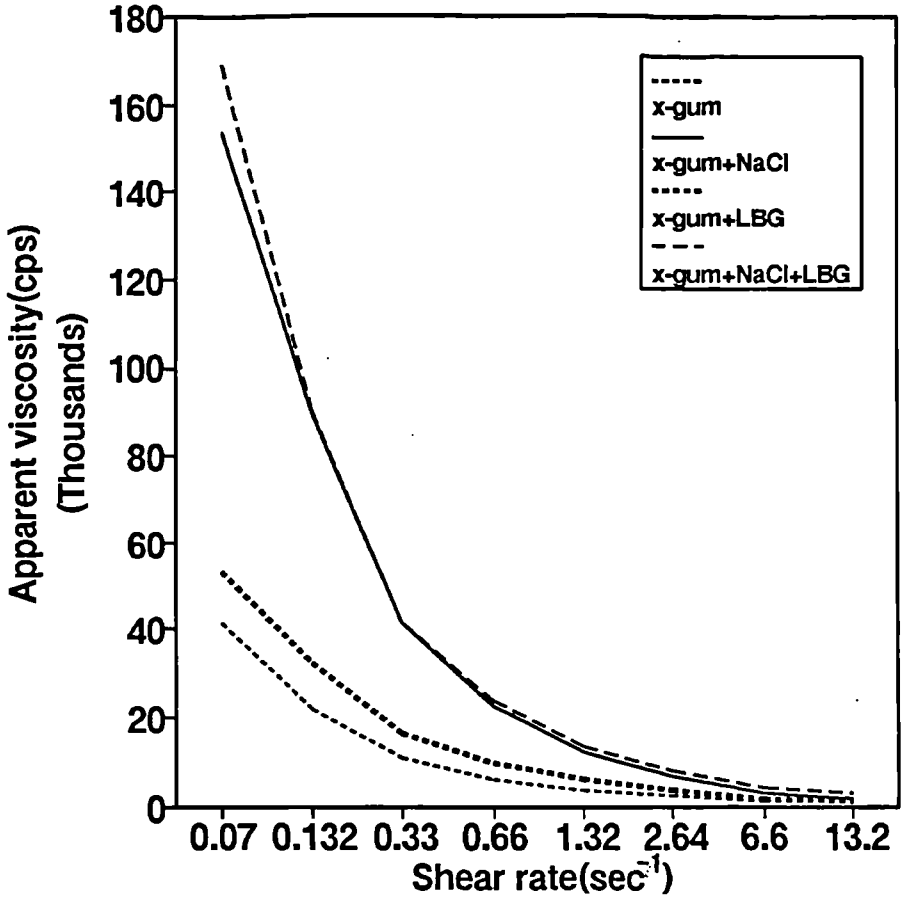


Figure 43: Measurement of apparent viscosity as a function of shear rate for air saturated (A) xanthan gum (1%) + mannitol (20%), (B) xanthan gum (1%), mannitol (20%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) and (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2}mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

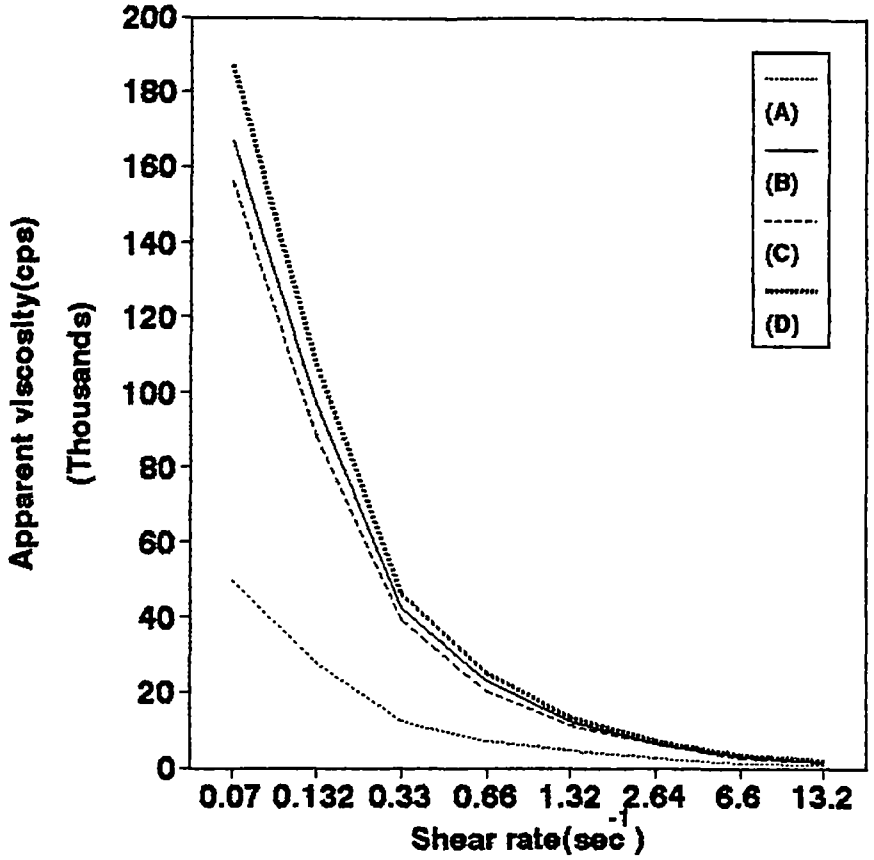


Figure 44: Measurement of apparent viscosity as a function of shear rate for air saturated (A) xanthan gum (1%) + mannitol (20%), and locust bean gum (LBG) 1%, (B) xanthan gum (1%), mannitol (20%) NaCl (0.1%) and LBG (1%), (C) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and LBG 1%) and (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}), NaCl (0.1%) and LBG (1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

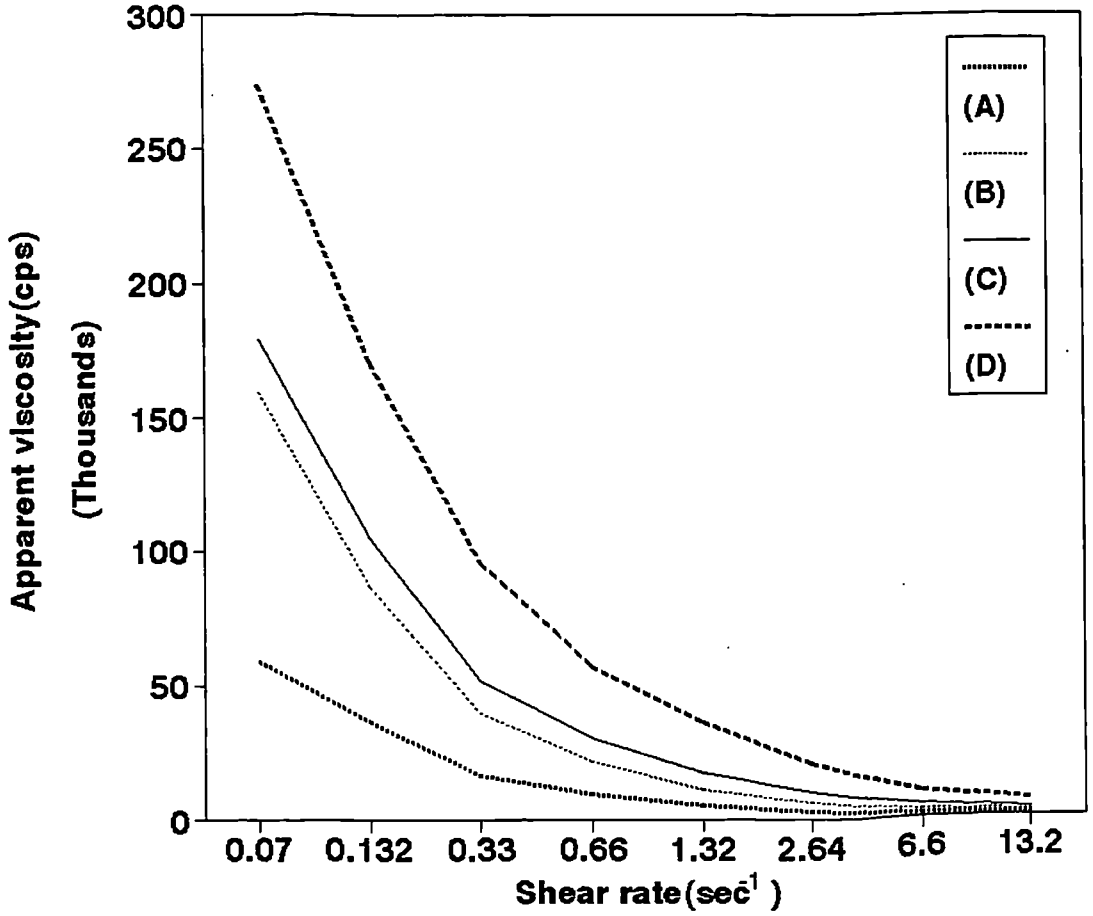
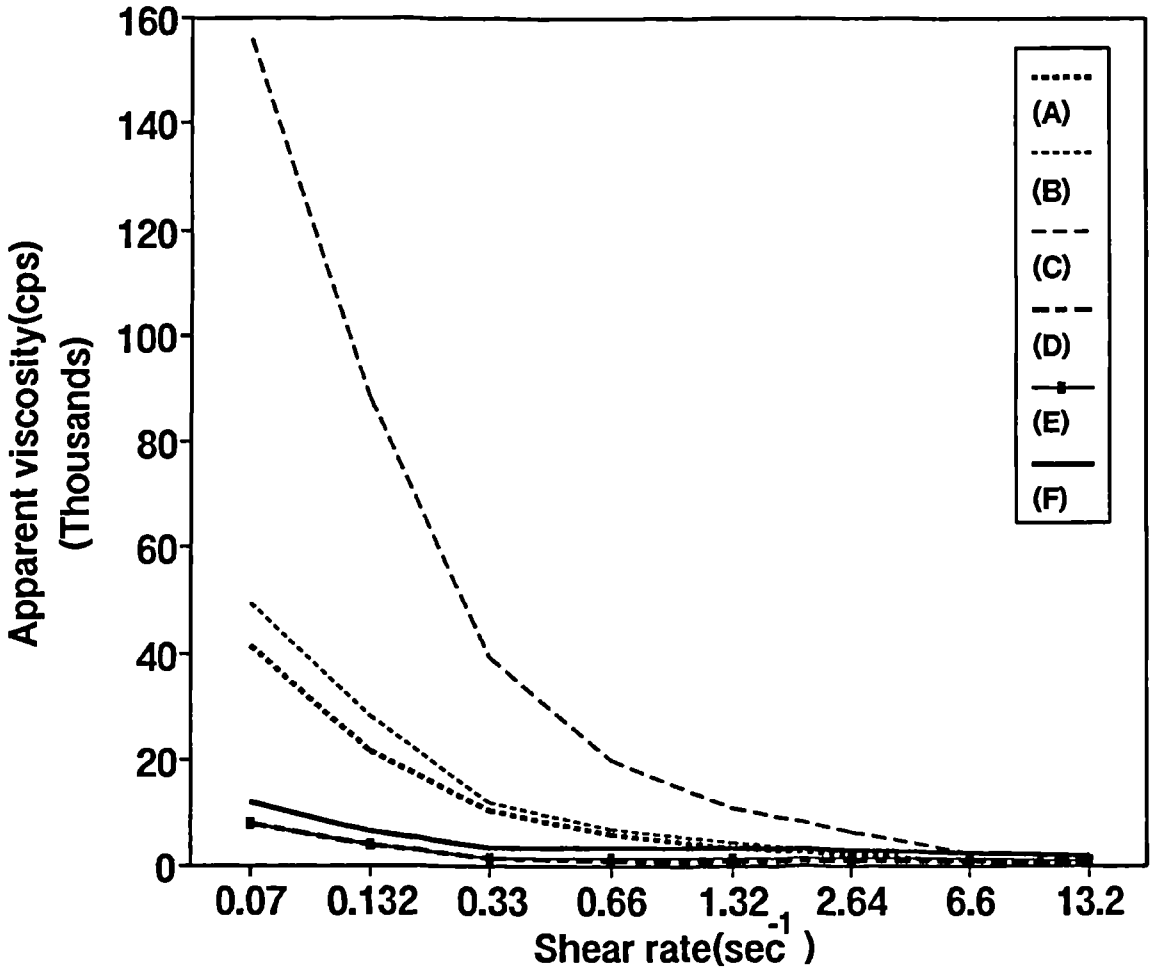


Figure 45: Measurement of apparent viscosity as a function of shear rate for (A) xanthan gum (1%), (B) xanthan gum (1%) + mannitol (20%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}), (D) locust bean gum (LBG) 1%, (E), LBG (1%) + mannitol (20%) and (F), LBG (1%) + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.



locust bean gum solutions the least.

Xanthan gums interact with galactomannans, such as locust bean gum[345]. The viscosity of mixtures of solutions containing 1% locust bean gum and 1% xanthan gum were measured as were similar solutions containing 20% mannitol alone and 20% mannitol and ascorbic acid (10^{-2} mol dm^{-3}), Figure 46.

In a separate experiment the order of mixing of the locust bean gum and xanthan gum were reversed and viscosity measurements again carried out in the presence and absence of mannitol and ascorbic acid as previously described (Figure 46). No significant differences are obtained and therefore the order of preparation of the mixture does not effect their final viscosities, (Figure 46).

At low shear rates the viscosity of xanthan gum and locust bean gum separately are ~40,000 and 10,000 CPS respectively (Figure 45) and the mixed solutions have an apparent viscosity of ~ 50,000 CPS (Figure 46), indicating that even though interaction between the polymers is proposed to occur this has no effect on the final apparent viscosity of the mixed solutions.

Similar data were obtained for the mixed solutions of xanthan and LBG containing mannitol alone and a mixture of mannitol/ascorbic acid. The final value of the viscosities appears to be sum of the viscosities of the individual components (Figure 47).

Further experiments were carried out using LBG/mannitol solution (1), xanthan gum/mannitol solution (2), LBG/mannitol/ascorbic acid solution (3) and xanthan gum/mannitol/ascorbic acid solution(4). Mixtures of solutions (1) and (2) gave a solution

Figure 46: Measurement of apparent viscosity as a function of shear rate for air saturated (A) locust bean gum (LBG) 1% + xanthan gum (1%), (B) LBG (1%) + mannitol (20%) and xanthan gum (1%), (C) LBG (1%) + mannitol (20%) +ascorbic acid (10^{-2} mol dm^{-3}) and xanthan gum (1%), (D) xanthan gum (1%) + LBG (1%), (E) xanthan gum (1%) + mannitol (20%) and LBG (1%) and (F) xanthan gum (1%) + mannitol (20%) + ascorbic acid (10^{-2} mol dm^{-3}) and LBG (1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

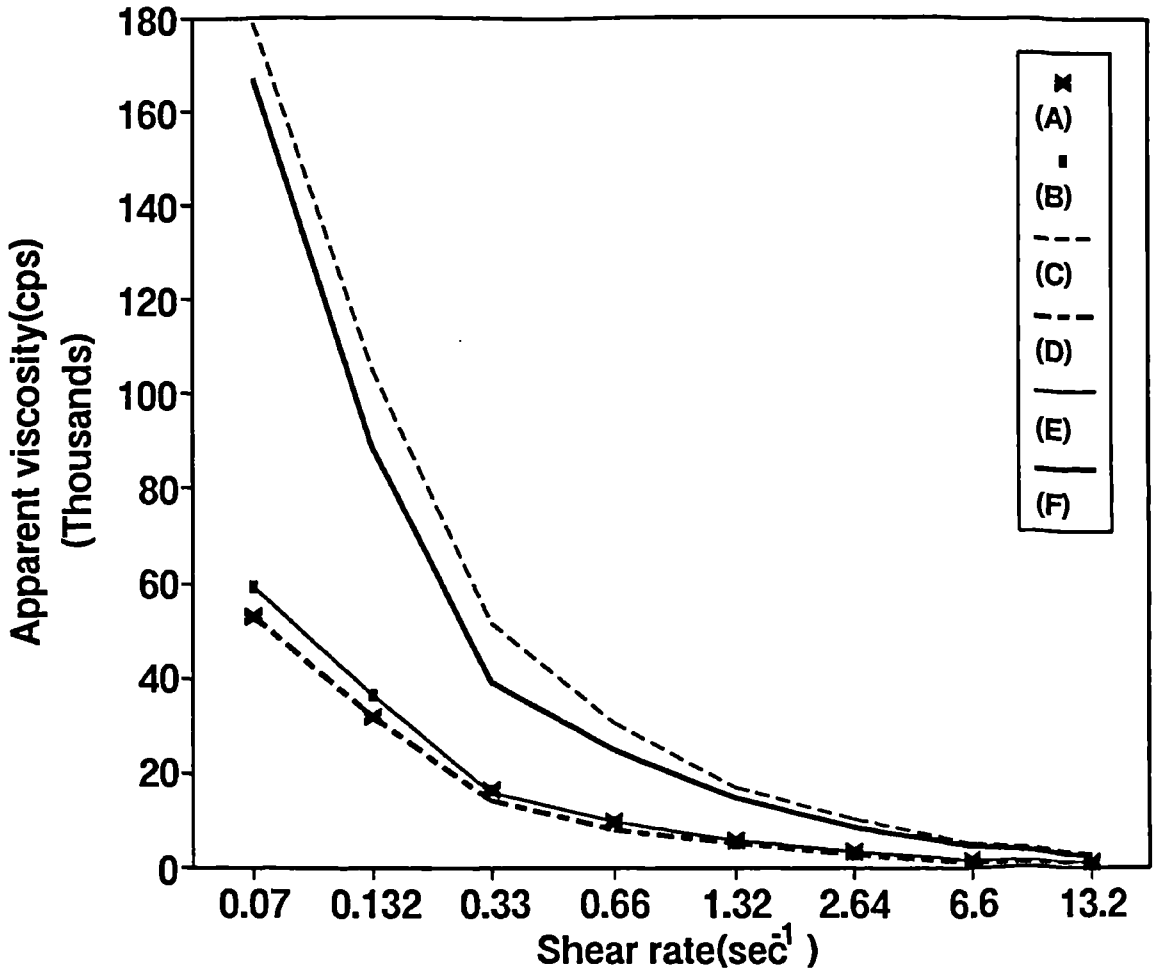
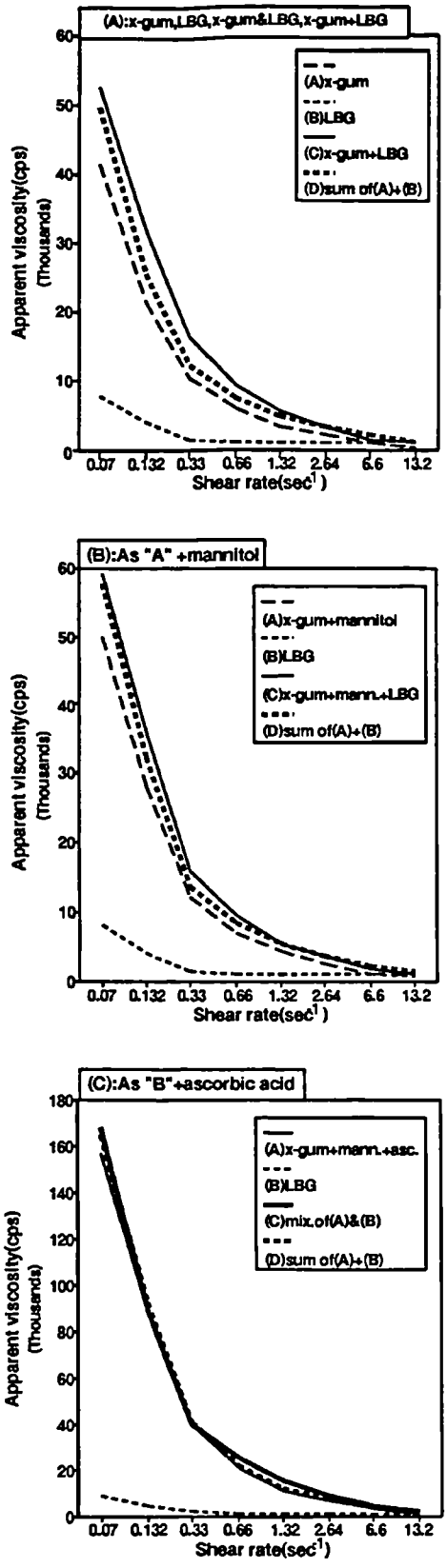


Figure 47: Comparison of apparent viscosity of (A) xanthan gum (1%), (B) LBG (1%), (C) LBG (1%) with combination of xanthan gum (1:1), (D) sum of (A) and (B), and in presence of mannitol (20%) or mannitol/ascorbic acid (10^{-2} mol dm^{-3}). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.



with viscosity ~ 150,000 CPS, [Figure 48(A)]. Mixtures of (1) and (4) [Figure 48(C)] and of (2) and (3) [Figure 48(B)] gave similar results, though the apparent viscosity increased ~ 1.5 fold compared with the solutions without ascorbic acid. Mixtures of (3) and (4) [Figure 48(D)] gave an apparent viscosity of ~ 300,000, a 2 fold increase compared with the solutions without ascorbic acid (Figure 48).

The data presented in figures (42 - 44) is for solutions containing air and similar data were obtained for solutions saturated with N_2 (Figure 49-51), N_2O (Figure 52) and N_2O/t-butanol (1 mol dm^{-3}), (Figure 53).

Xanthan gum solutions (1%) were irradiated in air and N_2 to increasing doses up to 25kGy and their apparent viscosity was again measured over the range of shear rates. The apparent viscosity in air saturated solutions of xanthan gum alone and xanthan gum/locust bean gum (LBG) mixture with or without NaCl (0.1%), using spindle number, 25 (speed 60rpm) is shown in Figure 54. Addition of NaCl (0.1%) to unirradiated solutions shows an increase in viscosity in both xanthan gum and xanthan gum in combination with LBG. At a dose of 25kGy (the sterilization dose) the apparent viscosity decreased to 2.15, 3.11, 31.6 and 45.8cps for xanthan gum, xanthan gum + NaCl, xanthan gum/LBG mixture and xanthan gum/LBG mixture containing NaCl respectively, indicating a very low viscosity after high irradiation dose. Figure 55 shows the plots of the apparent viscosity vs. shear rate for the same unirradiated and irradiated solutions as are given in Figure 54, at the various doses used. This data was obtained using the appropriate spindle at different shear rates, whereas using only spindle number 25, the appropriate spindle speed is 60rpm as in Figure 54.

The above experiment was repeated in the presence of N_2 (Figure 56 and 57). The decrease

Figure 48: Measurement of apparent viscosity of air saturated (A) locust bean gum (LBG) 1% + mannitol (20%) and xanthan gum (1%) + mannitol (20%), (B) LBG (1%) mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) + xanthan gum (1%) + mannitol (20%), (C) LBG (1%) + mannitol (20%) + xanthan gum (1%) + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) and (D) LBG (1%) + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) + xanthan gum (1%) + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

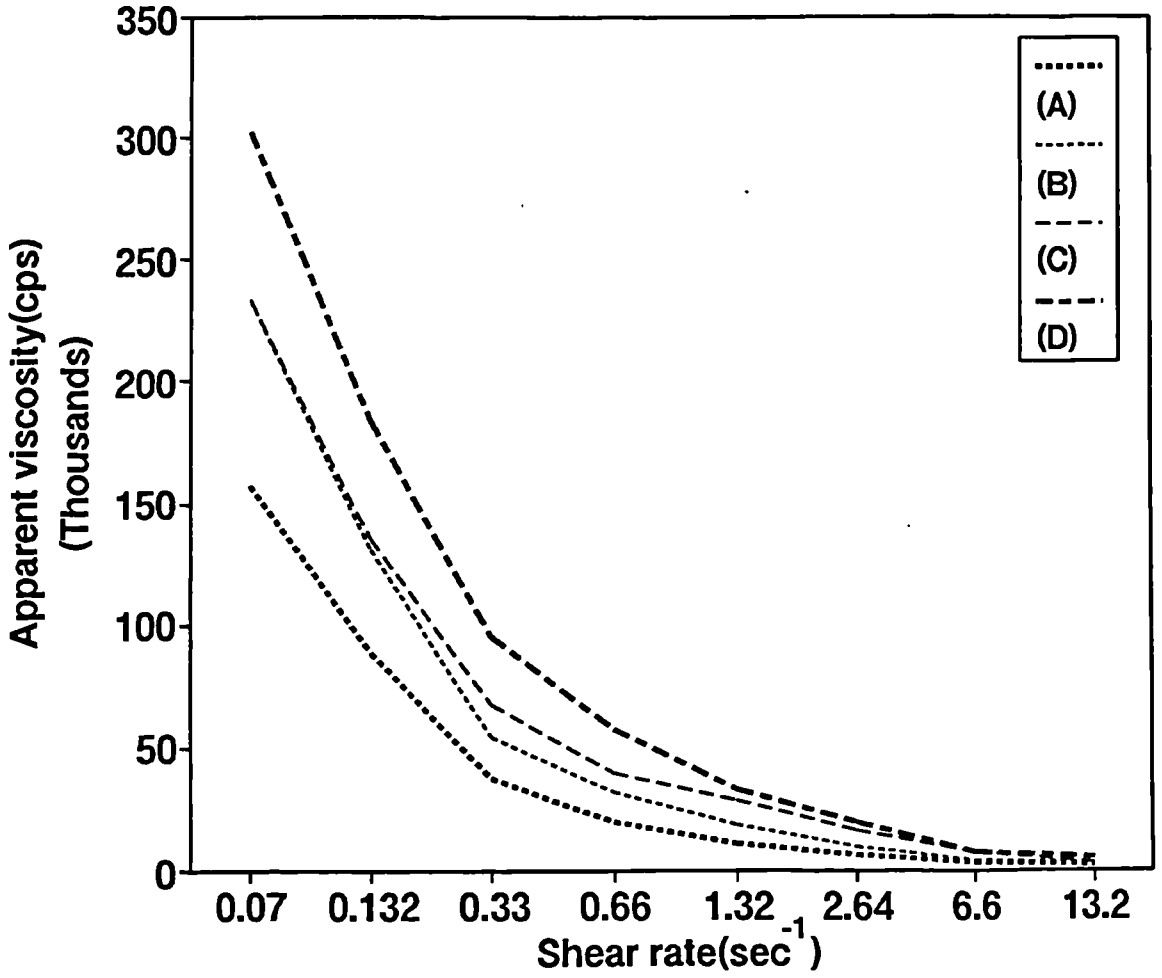


Figure 49: Effect of NaCl (0.1%) on the apparent viscosity of N_2-saturated xanthan gum/locust bean gum (1:1), measured using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

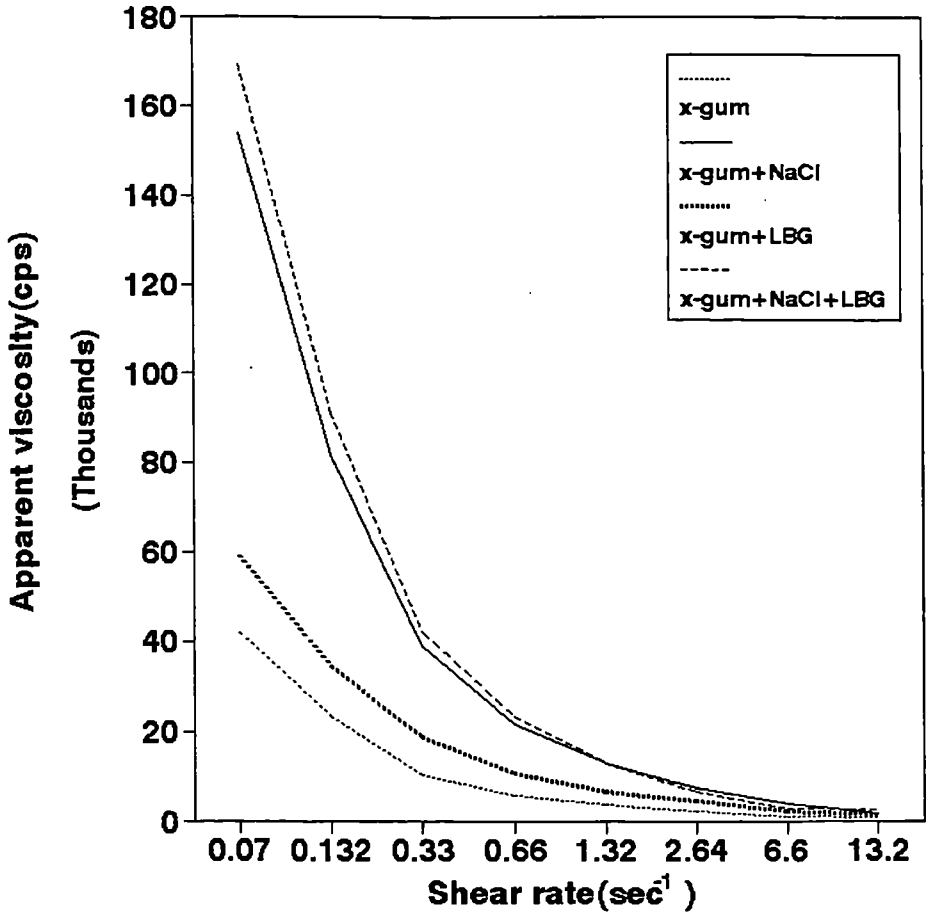


Figure 50: Measurement of apparent viscosity as a function of shear rate for N_2-saturated, (A) xanthan gum (1%) + mannitol (20%), (B) xanthan gum (1%), mannitol (20%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) and (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

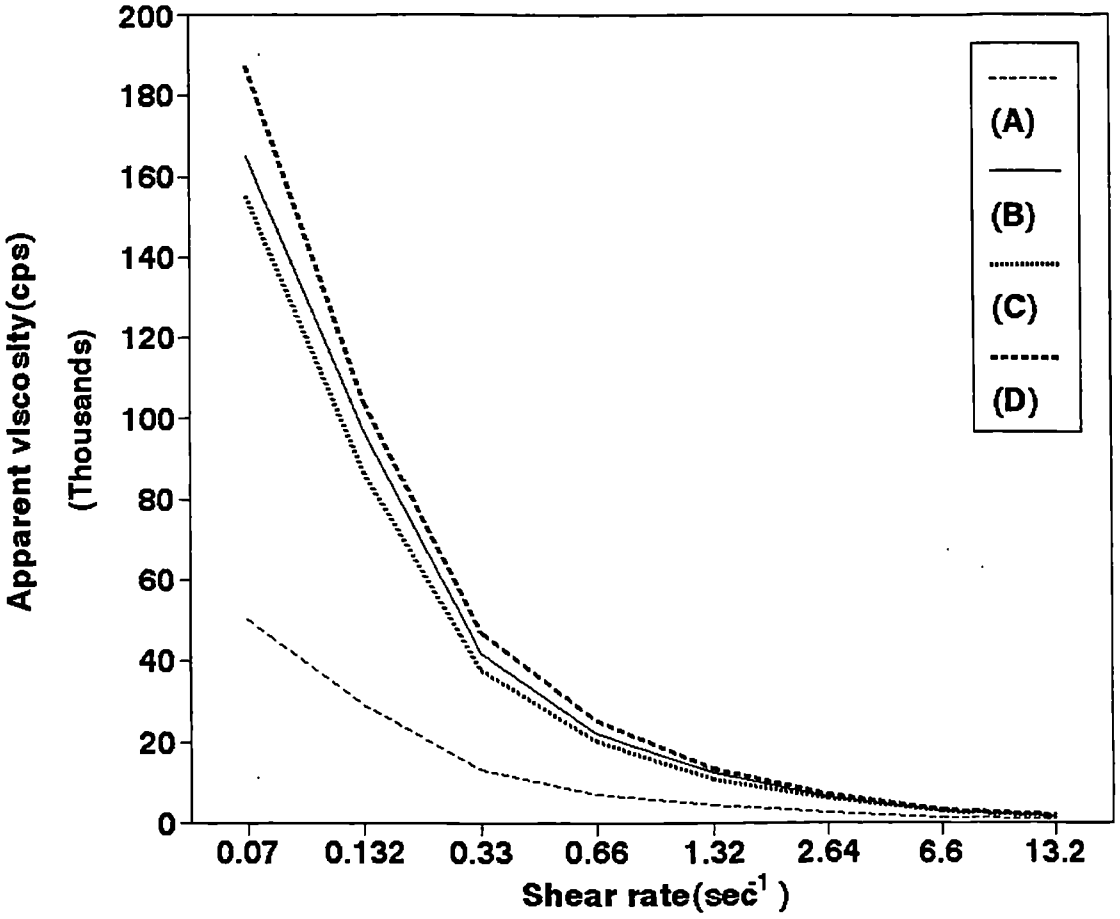


Figure 51: Measurement of apparent viscosity as a function of shear rate for N_2-saturated, (A) xanthan gum (1%) + mannitol (20%) and LBG (1%), (B) xanthan gum (1%), mannitol (20%), NaCl (0.1%) and LBG (1%), (C) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and LBG (1%) and (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}), NaCl (0.1%) and LBG (1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

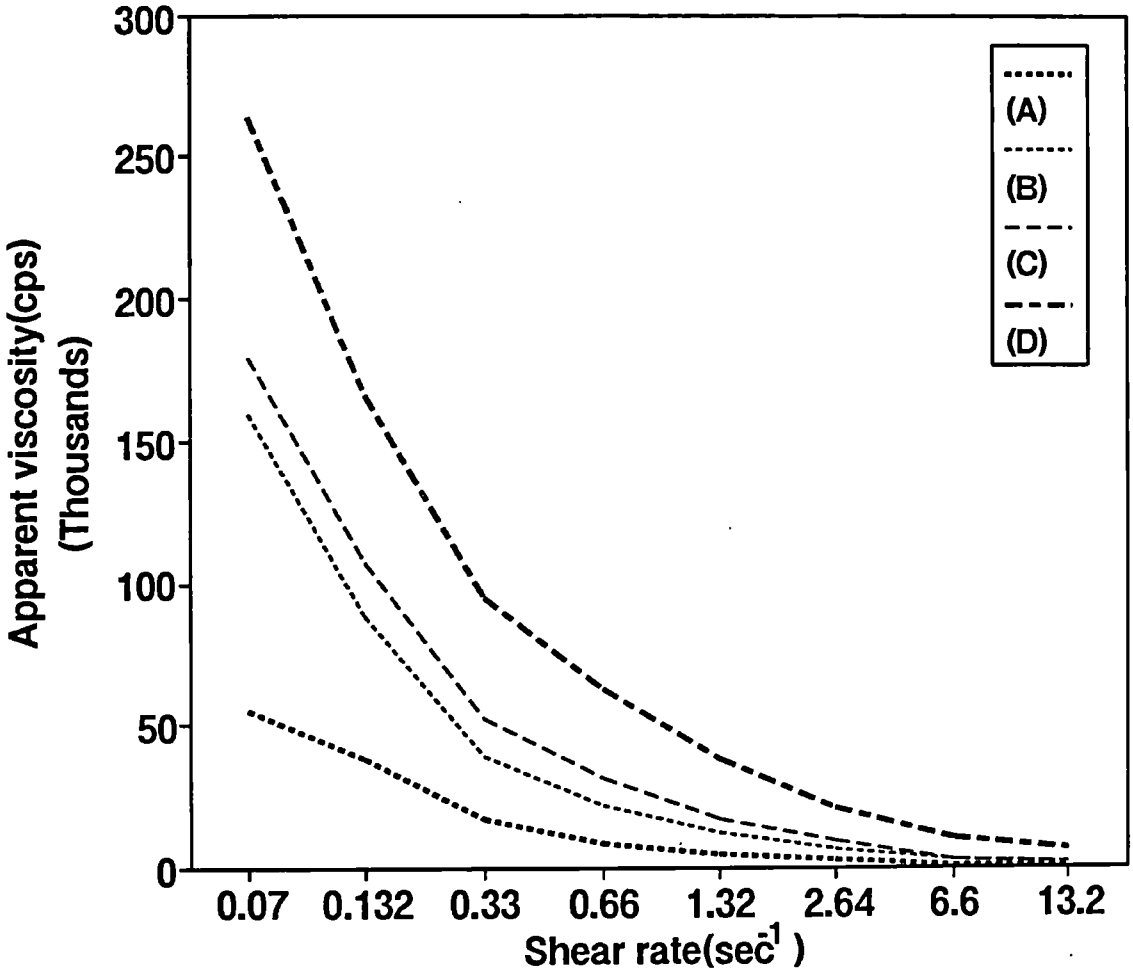


Figure 52: Apparent viscosity measurements of N_2O-saturated xanthan gum (1%) and in combination with locust bean gum (LBG) 1% and with NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

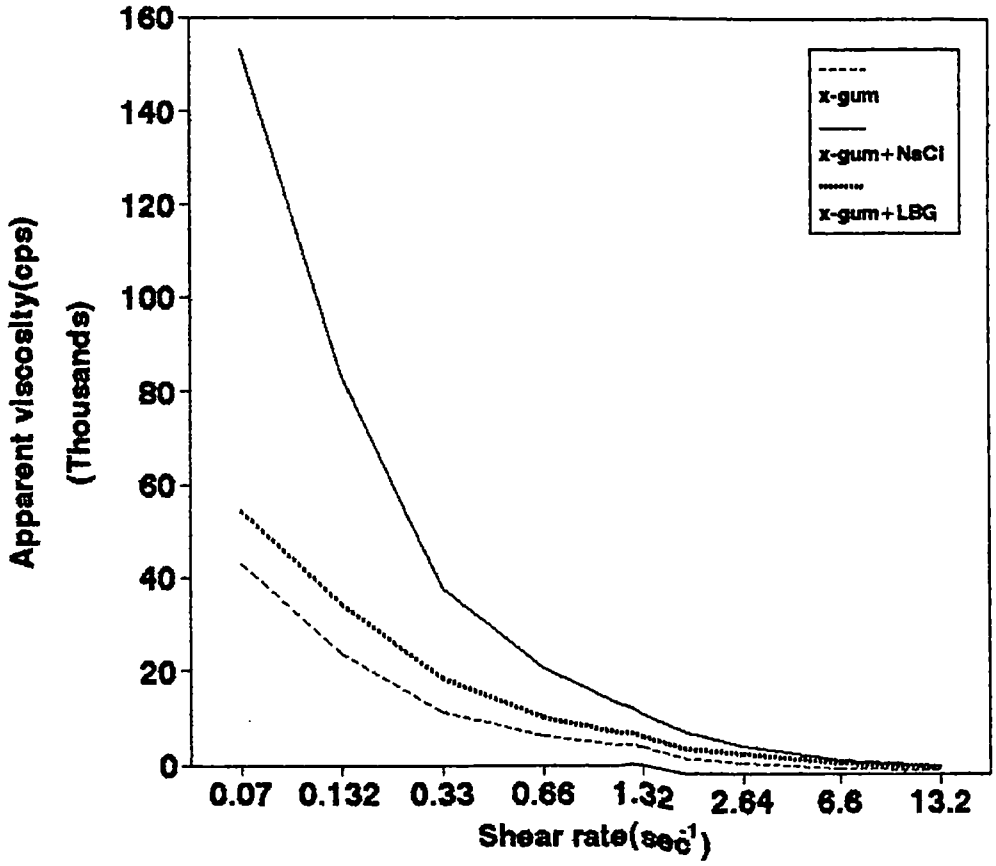


Figure 53: Apparent viscosity measurements of N_2O-saturated xanthan gum (1%) and in combination with locust bean gum (LBG) 1% and t-butanol (1 mol dm^{-3}), with NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

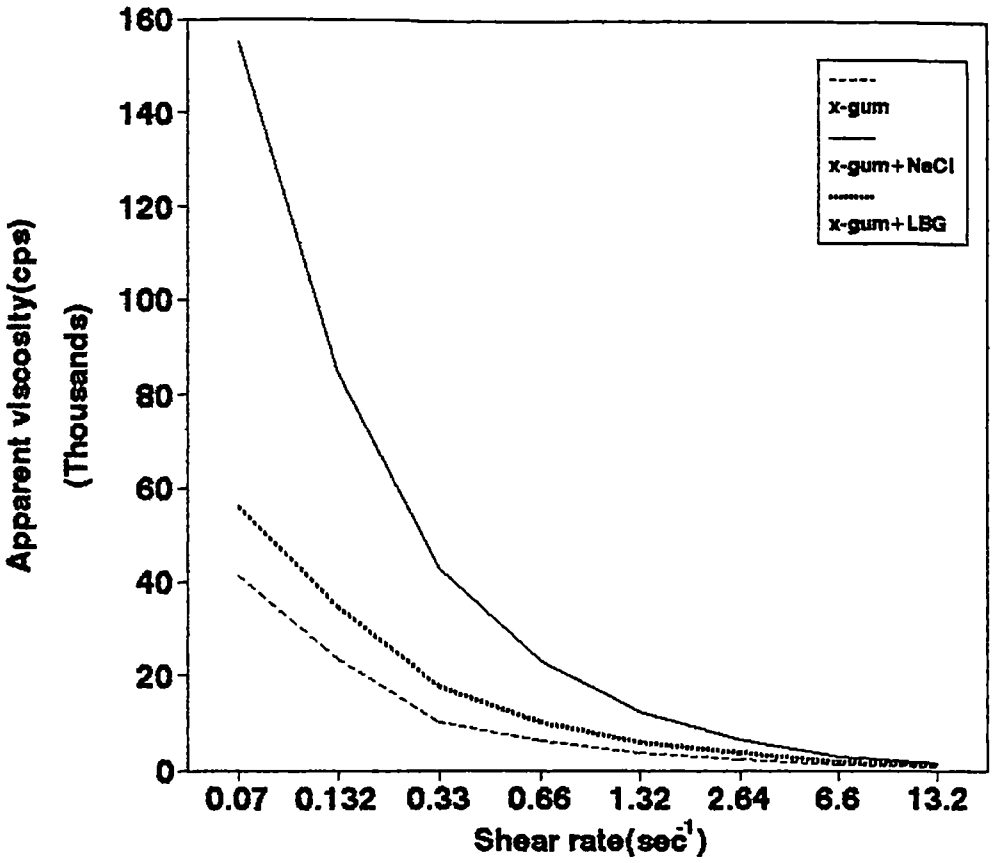


Figure 54: Effect of γ-irradiation on the apparent viscosity of air saturated xanthan gum/locust bean gum (LBG) 1%, in combination with or without NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60 rpm and spindle number 25, T = 20°.

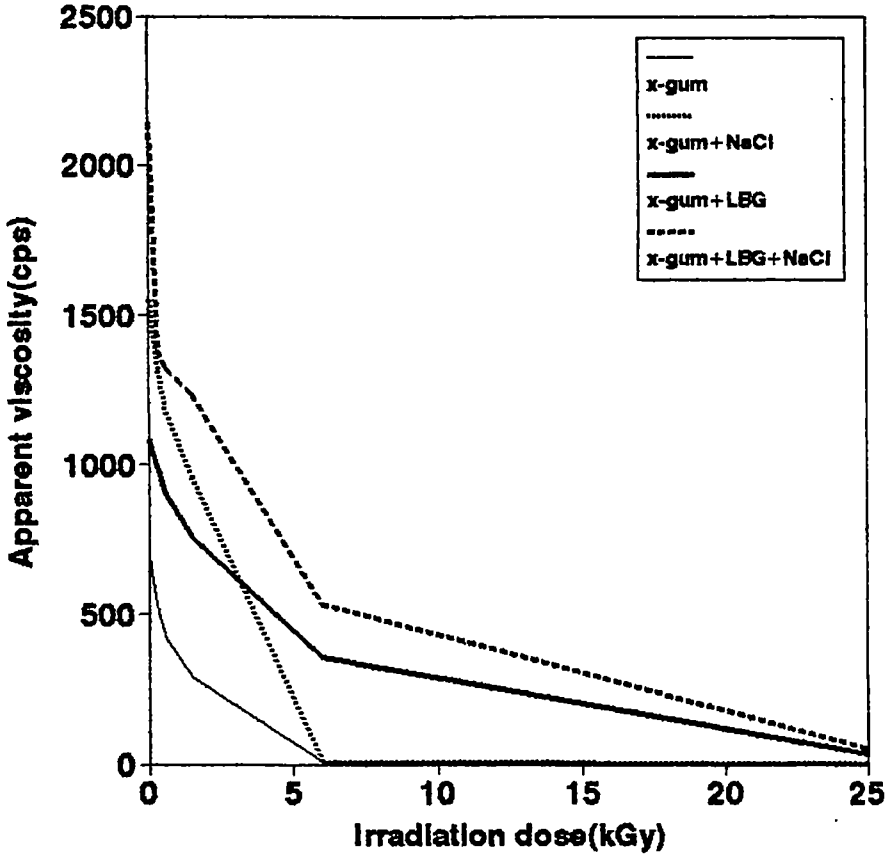
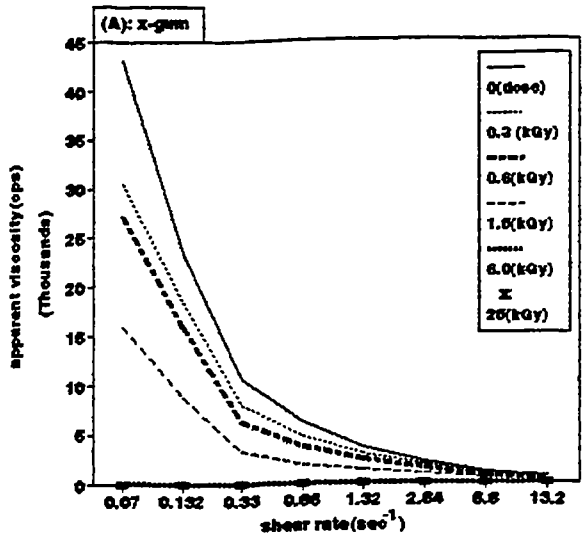
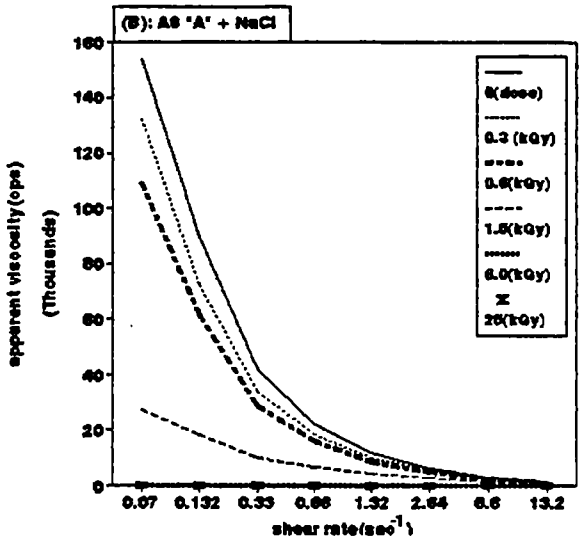
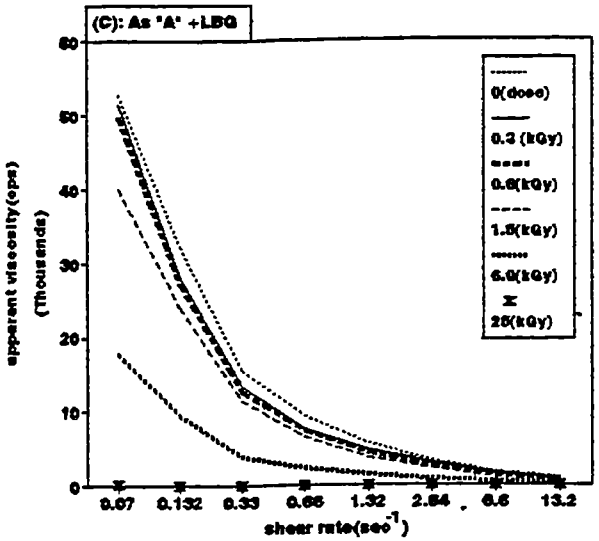


Figure 55: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of air saturated (A) xanthan gum (1%), (B) xanthan gum (1%) + NaCl (0.1%), (C) xanthan gum (1%) + locust bean gum (LBG) 1%, (D) xanthan gum (1%) + NaCl (0.1%) and locust bean gum (LBG) 1%. Using a Brookfield L.V.T. viscometer and the appropriate spindle. T = 20°.







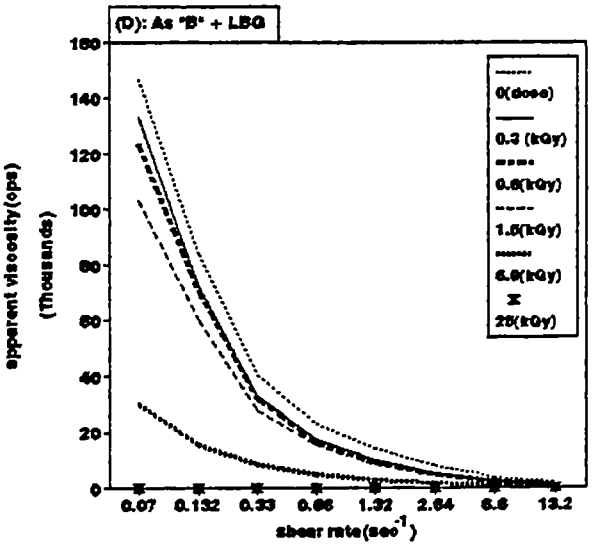


Figure 56: Effect of γ-irradiation on the apparent viscosity of N_2-saturated xanthan gum/locust bean gum (1:1) in combination with or without NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60rpm and spindle number 25, T = 20°.

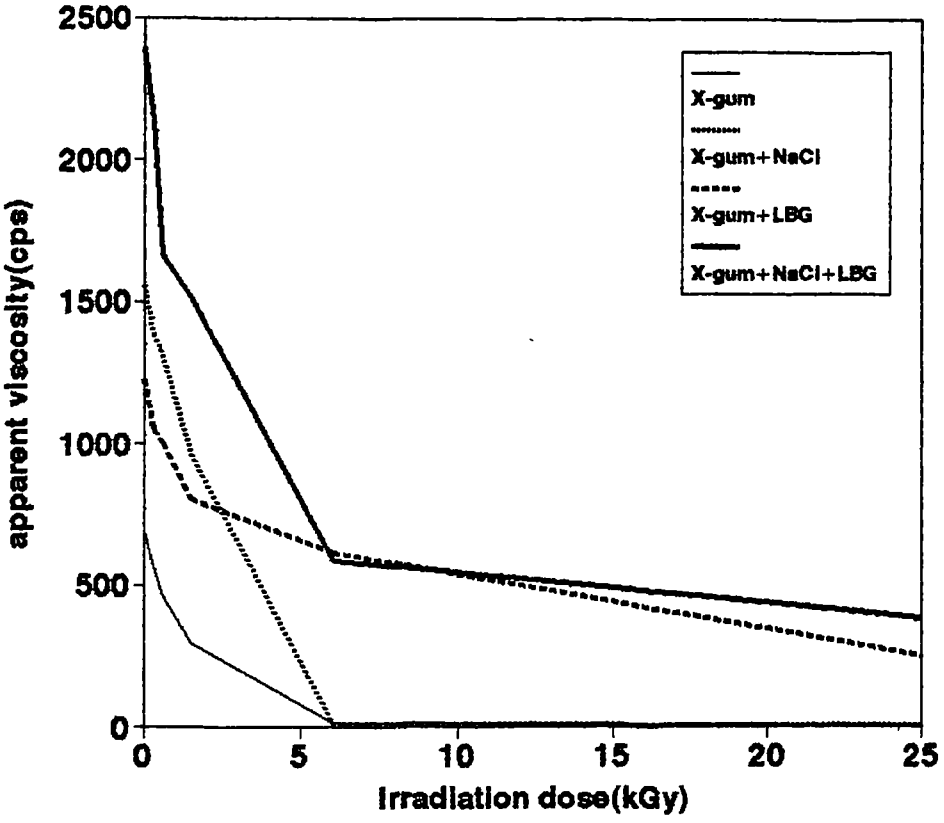
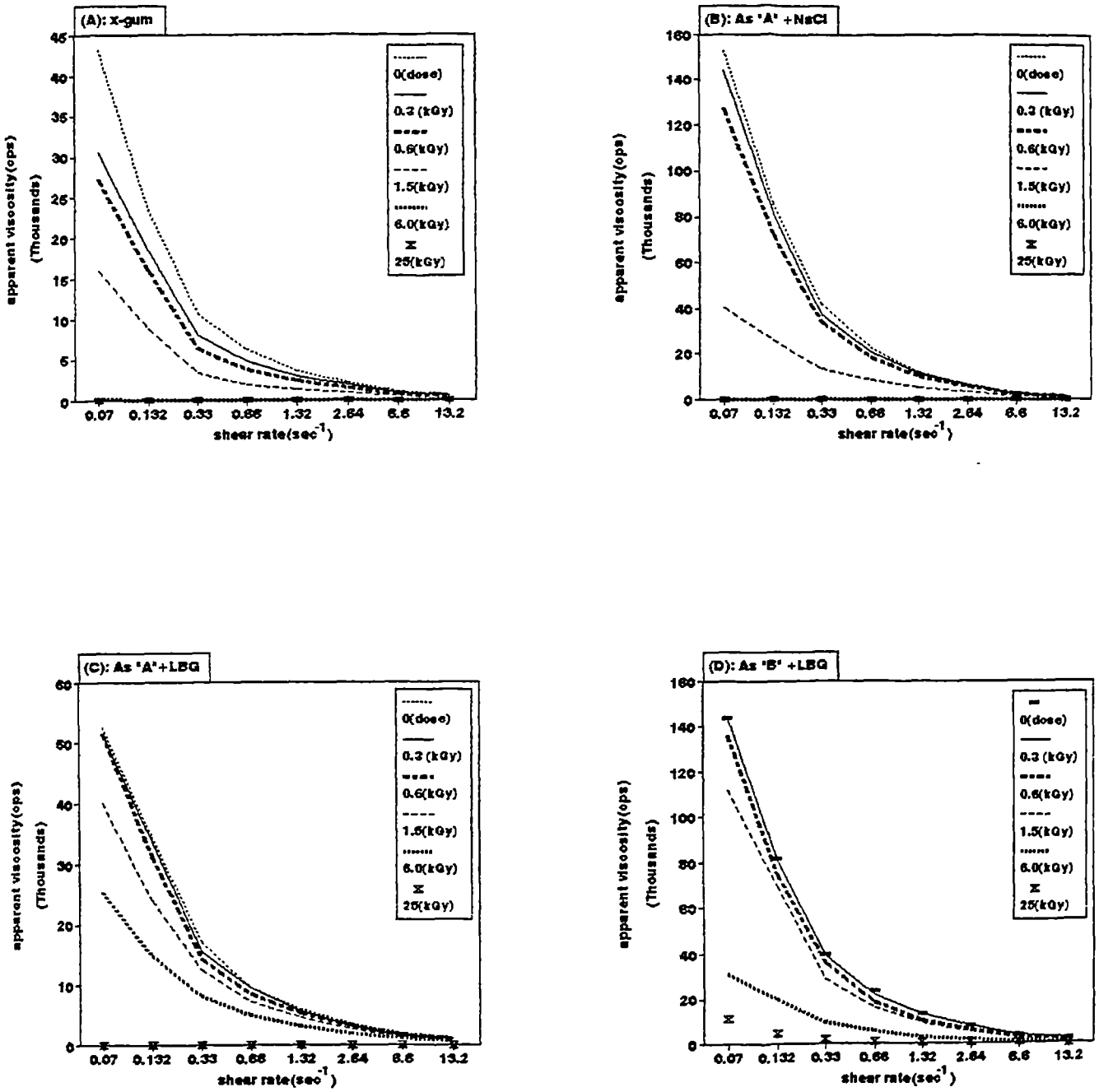


Figure 57: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of N_2 saturated (A) xanthan gum (1%), (B) xanthan gum (1%) + NaCl (0.1%), (C) xanthan gum (1%) + locust bean gum (LBG) 1% and (D) xanthan gum (1%) + NaCl (0.1%) and (LBG) 1%. Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.



of apparent viscosity at increasing radiation dose shows a similar pattern in air and N_2 saturated solutions, though for xanthan gum/LBG and xanthan gum//LBG + NaCl, the apparent viscosities show some increase in N_2 saturated solutions compared with those in air saturated solutions.

Addition of 20% mannitol to xanthan gum results in some stabilization to radiation of the solution, and significant viscosities can be measured up to doses of 25kGy. The apparent viscosity vs. dose plots and apparent viscosity vs. shear rate plots using spindle 25, are given in Figure 58 and 59. The same solution (ie. 20% mannitol/xanthan gum) was also irradiated in the presence of NaCl (0.1%) alone, ascorbic acid (10^{-2} mol dm^{-3}) alone and in the presence of NaCl and ascorbic acid. This data is also presented in Figure 58 and 59. The highest viscosity of the irradiated solutions was found in solutions containing xanthan gum + mannitol + ascorbic acid + NaCl.

The same solutions as described in Figure 59 were also irradiated in combination of locust bean gum (LBG), (Figure 60 and 61). The final viscosities after irradiation to 25 kGy of solutions containing ascorbic acid (Figure 60, C and D), are greater than those without ascorbic acid as was observed in Figure 58, C and D.

Radiolysis of the xanthan gum solutions of similar composition as are described in Figure 58 and 59 were carried out in the presence of N_2. No substantial differences are observed whether irradiation is carried out in air of N_2 (Figures 62 and 63).

Solutions described in Figures 60 and 61 were also irradiated in presence of N_2 to increasing doses (up to 25kGy), and their apparent viscosity again showed no substantial differences

Figure 58: Effect of γ-irradiation on the apparent viscosity of air saturated (A) xanthan gum (1%) + mannitol (20%), (B) xanthan gum (1%), mannitol (20%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}), (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60rpm and spindle number 18, T = 20°.

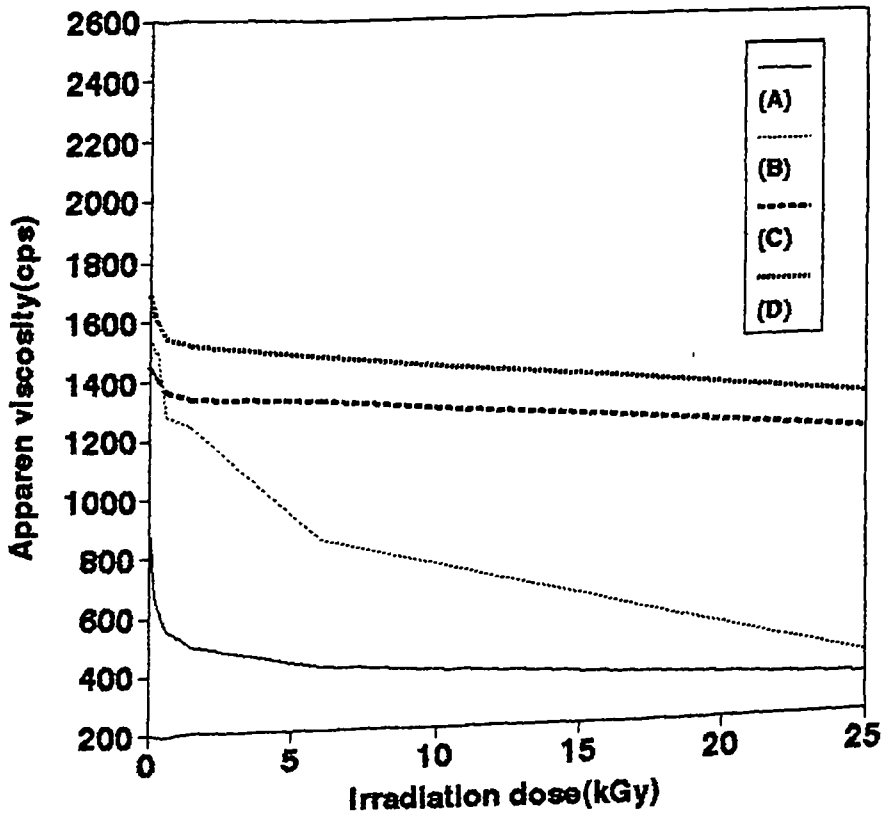
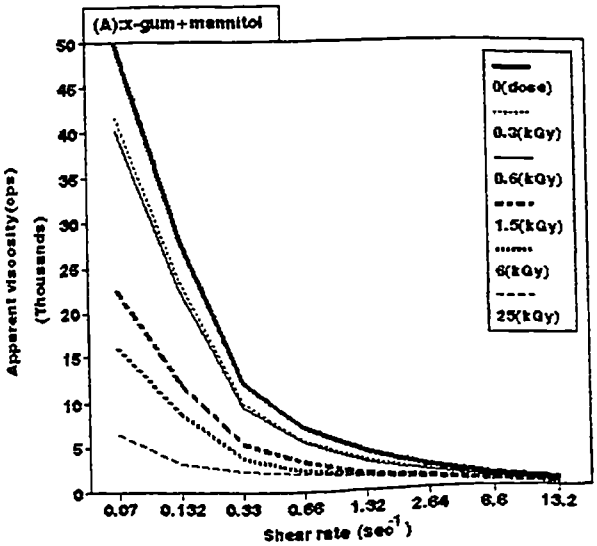
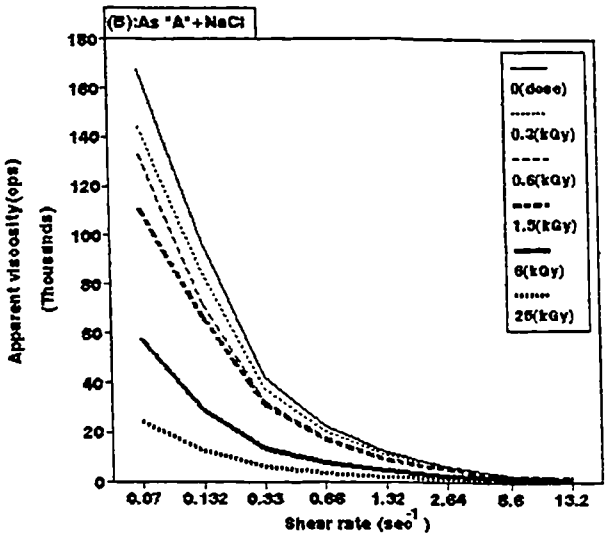
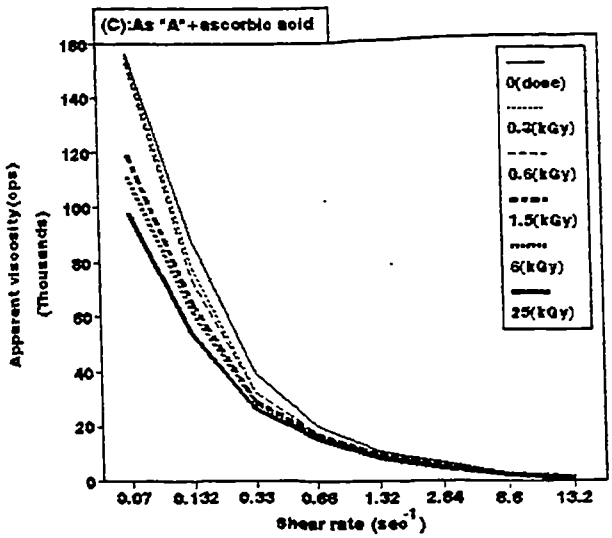


Figure 59: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of air saturated (A) xanthan gum (1%) + mannitol (20%), (B) xanthan gum (1%), mannitol (20%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}), (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.







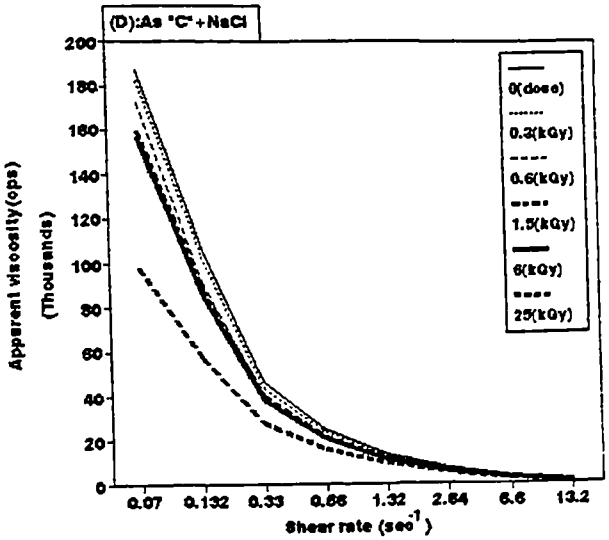


Figure 60: Effect of γ-irradiation on the apparent viscosity of air saturated (A) xanthan gum (1%), mannitol (20%) and locust bean gum (LBG) 1%, (B) xanthan gum (1%), mannitol (20%), LBG (1%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%), LBG (1%) and ascorbic acid (10^{-2} mol dm^{-3}), (D) xanthan gum (1%), mannitol (20%), LBG (1%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60 rpm spindle number 25, T = 20°.

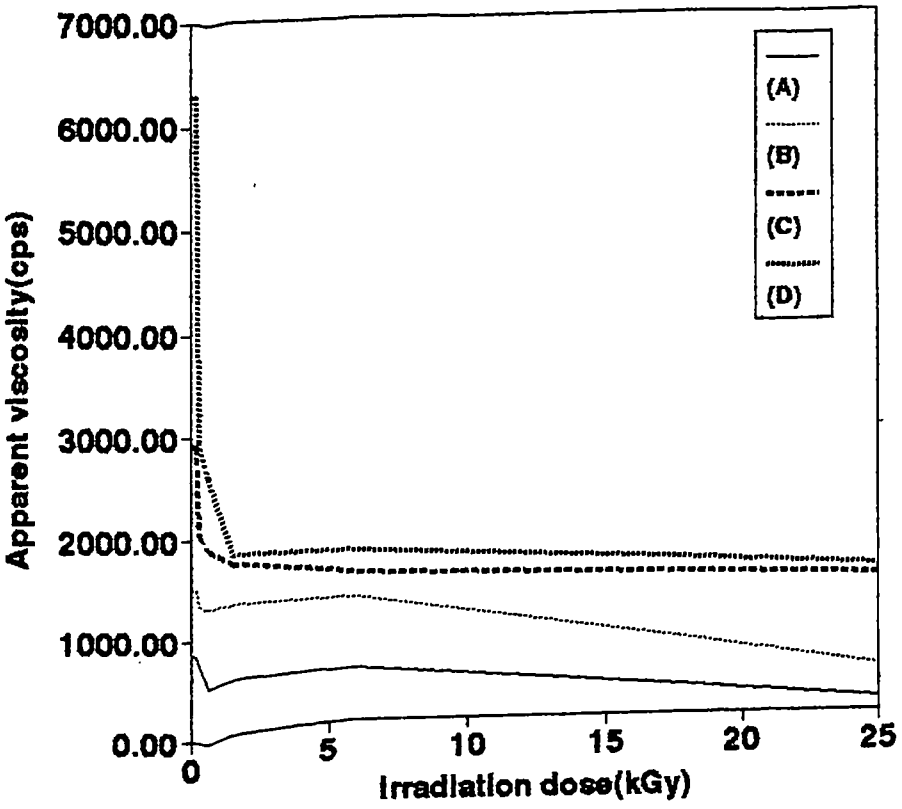


Figure 61: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of air saturated, (A) xanthan gum (1%), mannitol (20%) and locust bean gum (LBG) 1%, (B) xanthan gum (1%), mannitol (20%), NaCl (0.1%) and LBG (1%), (C) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and LBG (1%), (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}), NaCl (0.1%) and LBG (1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

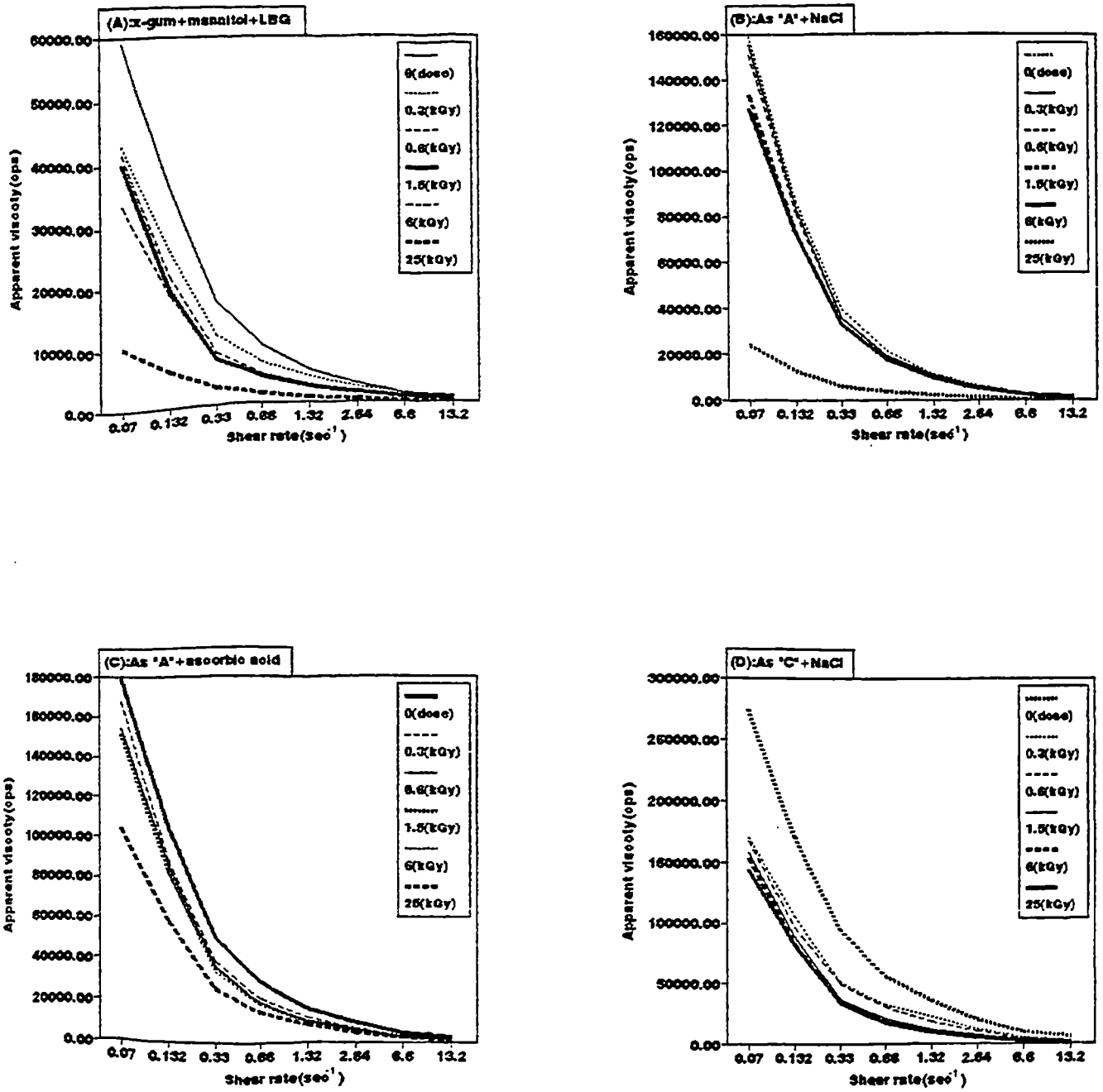


Figure 62: Effect of γ-irradiation on the apparent viscosity of N_2-saturated, (A) xanthan gum (1%) + mannitol (20%), (B) xanthan gum (1%), mannitol (20%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}), (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60rpm spindle number 25, T = 20°.

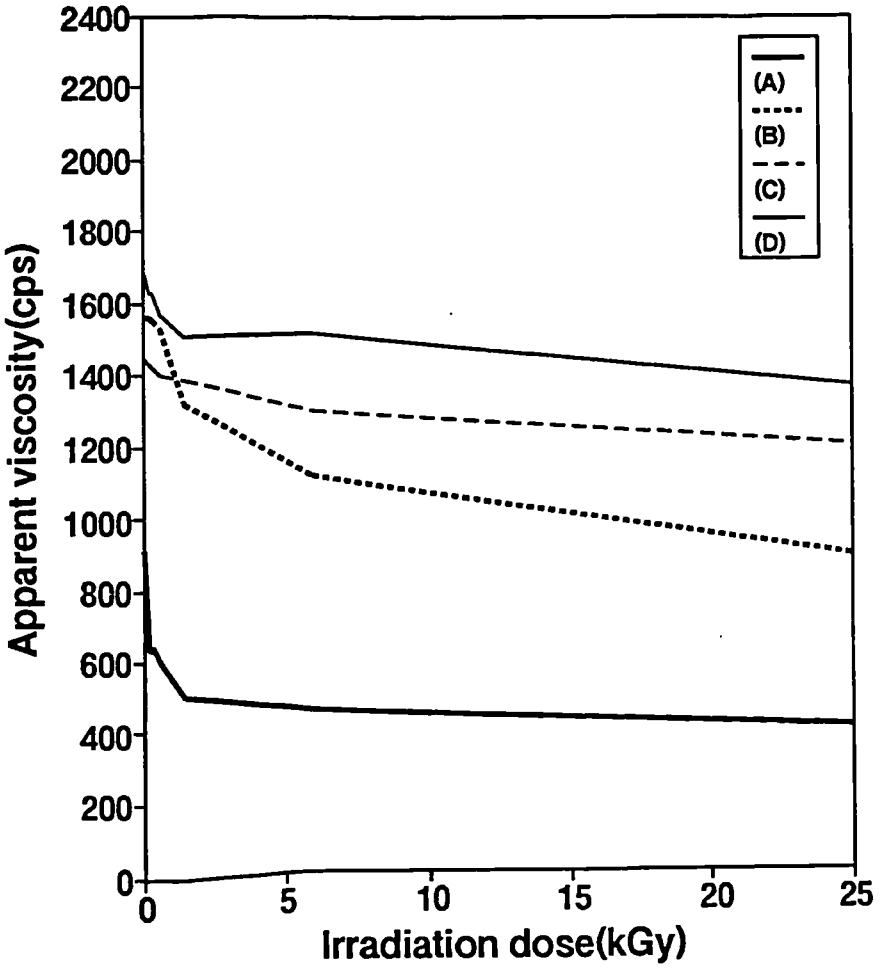
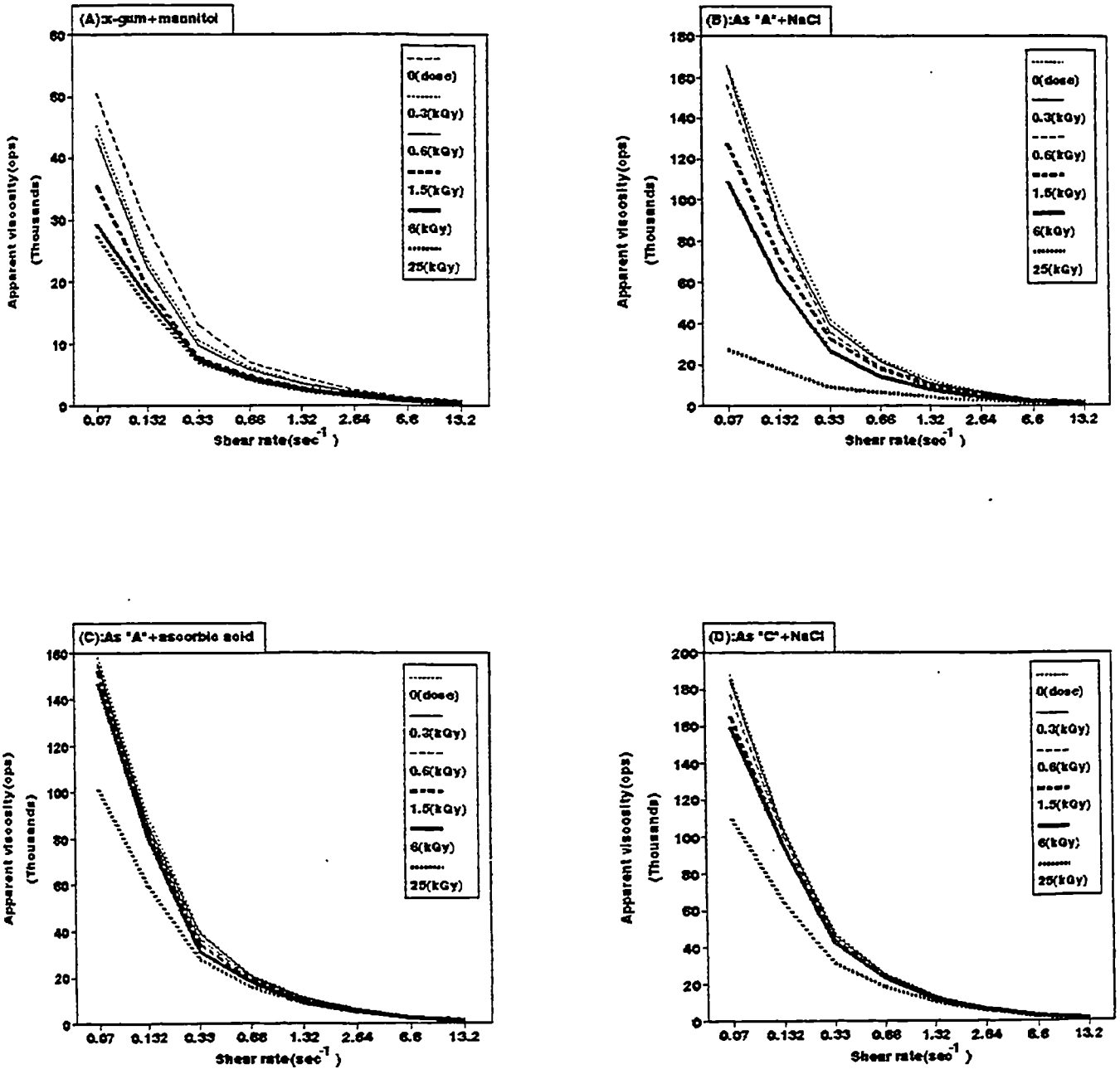


Figure 63: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of N_2-saturated (A) xanthan gum (1%) + mannitol (20%), (B) xanthan gum (1%), mannitol (20%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.



compared with those same solutions (Figure 64 A, B and C) irradiated in air. However solution (D) of (Figure 60) irradiated in air showed a greater decrease compared with the same solution (Figure 64 D) irradiated in N_2, (Figures 64 and 65).

Xanthan gum solutions (1%) in combination with LBG (1:1) and with NaCl (0.1%) were also irradiated in N_2O to increasing doses (up to 25kGy) and their apparent viscosity again measured over the range of shear rates. The results indicate that the initial rate of decrease in viscosity for solutions irradiated in N_2O is greater than that found for N_2 and air saturated solutions (Figure 66 and 67).

The decrease in apparent viscosity of the above solutions irradiated in the presence of N_2O and t-butanol (1 mol dm^{-3}), (Figure 68 and 69) is not as great as that found for the N_2O saturated solutions given in Figure (66 and 67).

Solutions of locust bean gum (LBG) with or without mannitol (20%) and with mannitol, ascorbic acid (10^{-2} mol dm^{-3}) were irradiated up to 25kGy and their apparent viscosity measured over a range of shear rates were compared with that for unirradiated solutions. It was found that as the shear rate increases the apparent viscosity decreases indicating again non-Newtonian (Pseudoplastic) behaviour.

Their viscosities also decreased markedly when the solutions were irradiated to a dose of 25 kGy (Figure 70). The above solutions were also mixed with the addition of xanthan gum (1%) and the results again showed some increase of the apparent viscosity up to a dose of 25 kGy (Figure 71) compared with solutions without xanthan gum.

Figure 64: Effect of γ-irradiation on the apparent viscosity of N_2 saturated (A) xanthan gum (1%), mannitol (20%) and locust bean gum (LBG) 1%, (B) xanthan gum (1%), mannitol (20%), LBG (1%) and NaCl (0.1%), (C) xanthan gum (1%), mannitol (20%), LBG (1%), ascorbic acid (10^{-2} mol dm^{-3}), (D) xanthan gum (1%), mannitol (20%), LBG (1%), ascorbic acid (10^{-2} mol dm^{-3}) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60rpm spindle number 25, T = 20°.

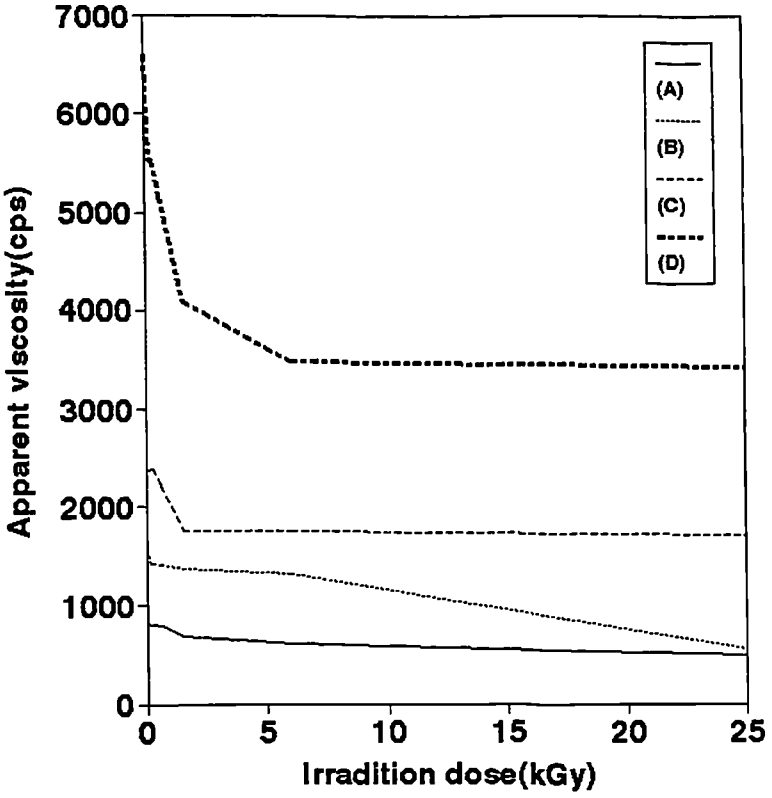


Figure 65: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of N_2-saturated (A) xanthan gum (1%), mannitol (20%) and LBG (1%), (B) xanthan gum (1%), mannitol (20%), NaCl (0.1%) and LBG (1%), (C) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and LBG (1%), (D) xanthan gum (1%), mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}), NaCl (0.1%) and LBG (1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

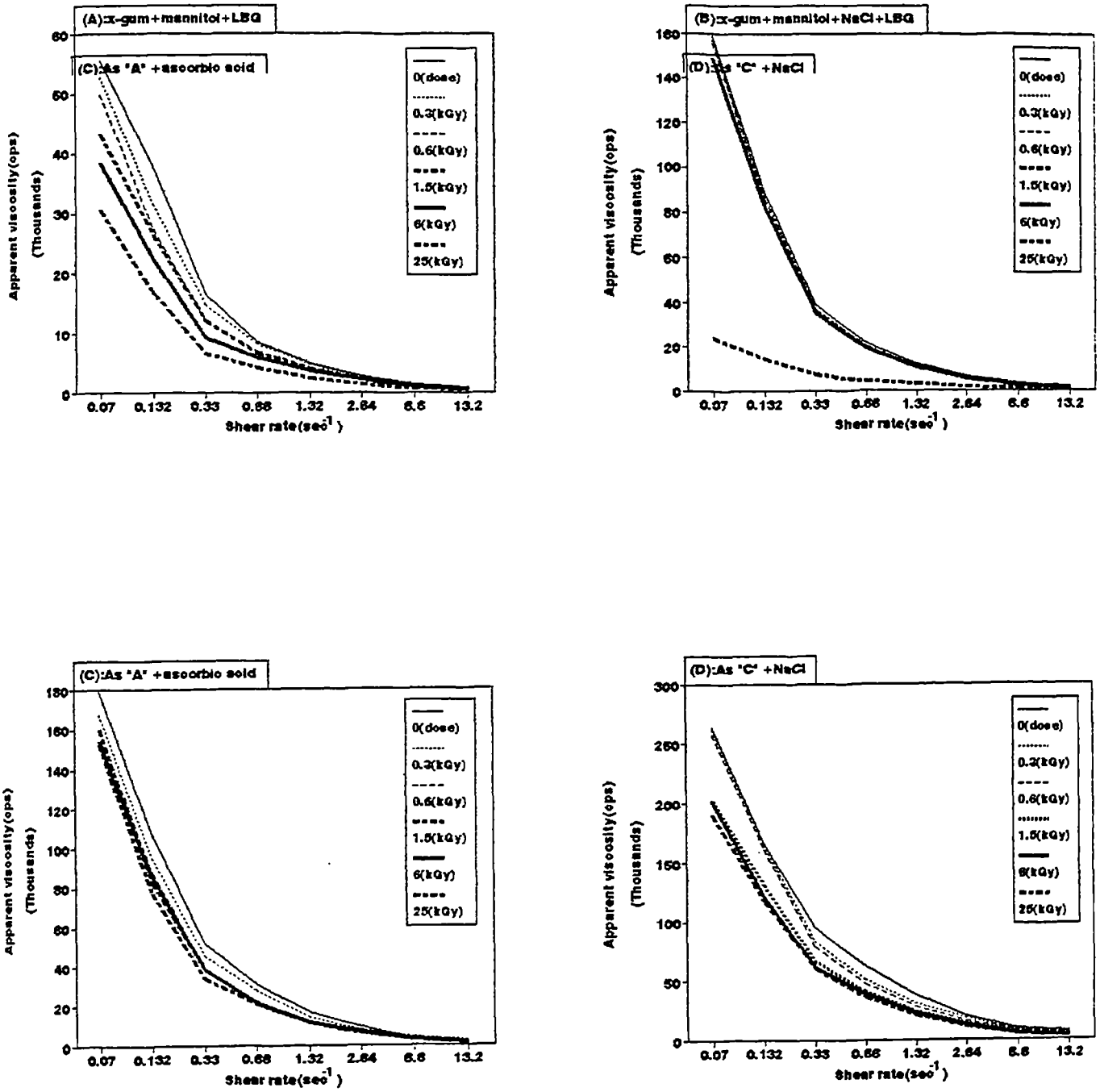


Figure 66: Effect of γ-irradiation on the apparent viscosity of N_2O-saturated xanthan gum (1%), xanthan gum (1%) + NaCl (0.1%) and xanthan gum (1%) + LBG (1%). Using a Brookfield L.V.T. viscometer at 60 rpm and spindle number 25, T = 20°.

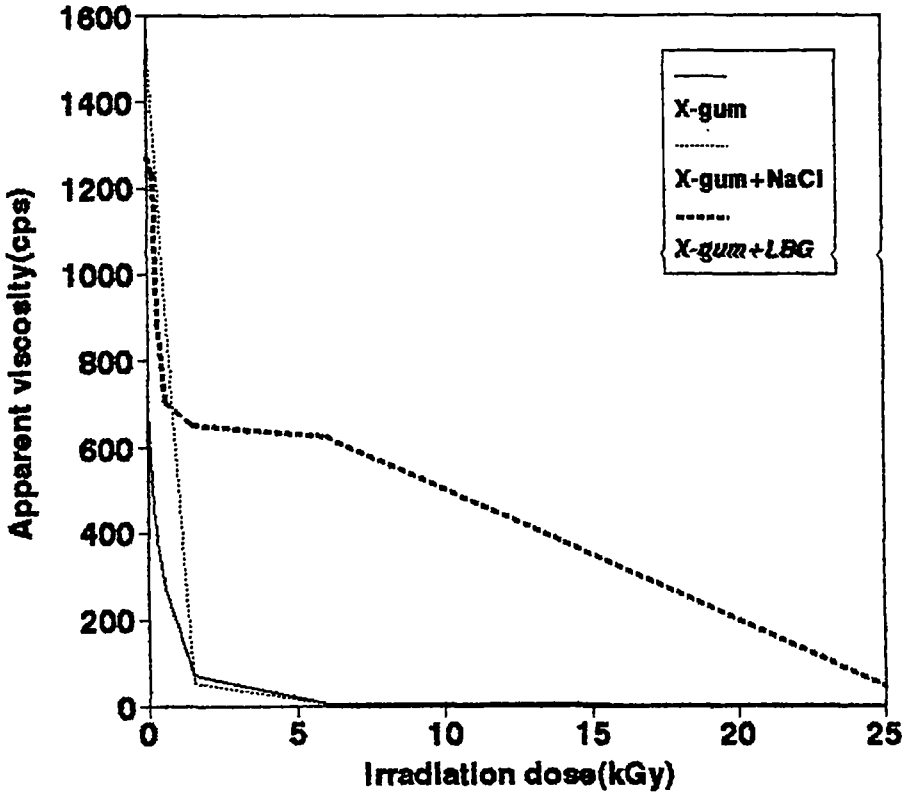


Figure 67: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated of N_2O saturated xanthan gum (1%), xanthan gum (1%) + NaCl (0.1%), xanthan gum (1%) + LBG (1%). Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.

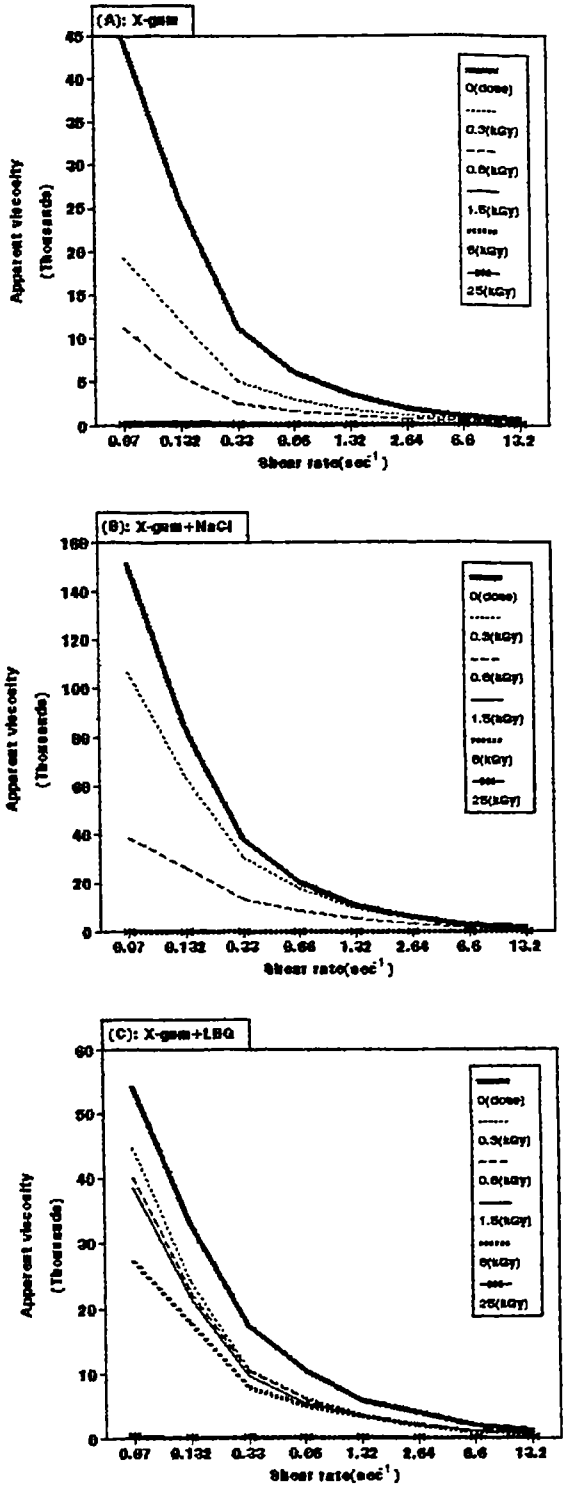


Figure 68: Effect of γ-irradiation on the apparent viscosity of N_2O saturated xanthan gum (1%) and in combination with locust bean gum (LBG) 1% and t-butanol (1 mol dm^{-3}), with or without NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60 rpm spindle number 25, T = 20°.

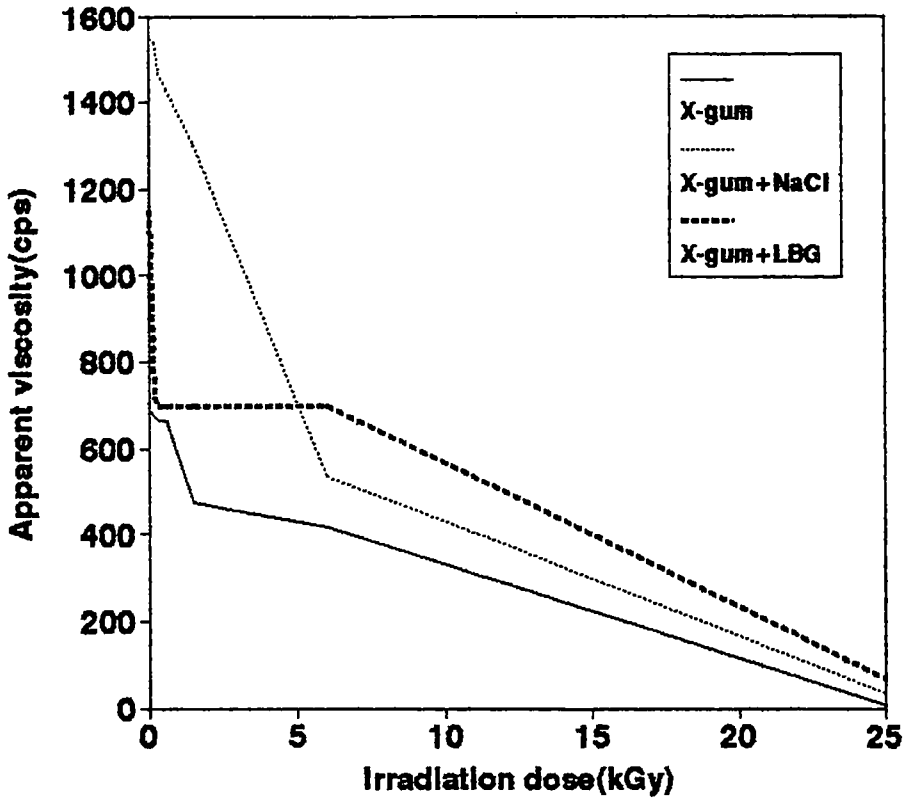


Figure 69: Apparent viscosity measurements for unirradiated/irradiated of N_2O-saturated xanthan gum (1%) and in combination with LBG (1%) and t-butanol (1 mol dm^{-3}), with or without NaCl (0.1%). Using a Brookfield L.V.T. viscometer, spindle number 25, T = 20°.

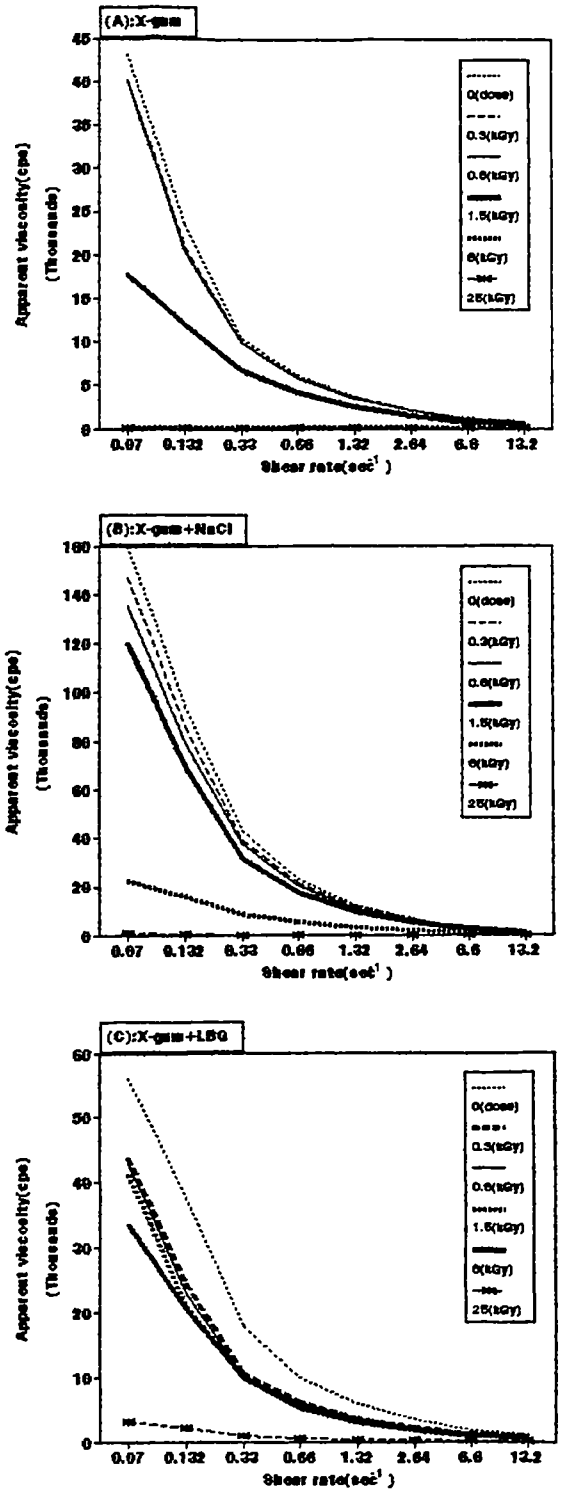
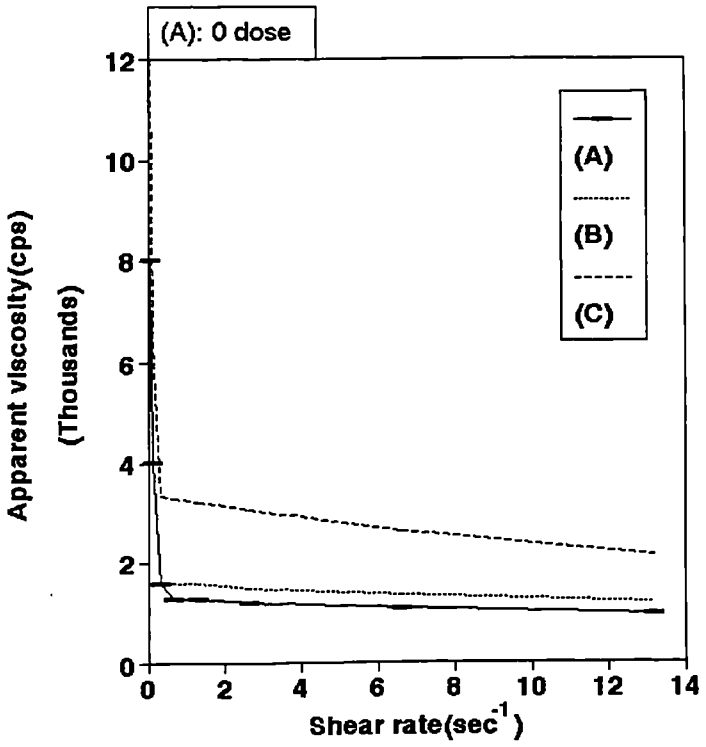


Figure 70: Apparent viscosity (cps) vs. shear rate (sec^{-1}) for unirradiated/irradiated up to sterilized dose (25kGy) of air saturated (A) LBG (1%), (B) locust bean gum (LBG) 1% + mannitol (20%) and (C) LBG (1%) + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}), (D) as (A) but sterilized, (E) as (B) but sterilized and (F) as (C) but sterilized. Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.



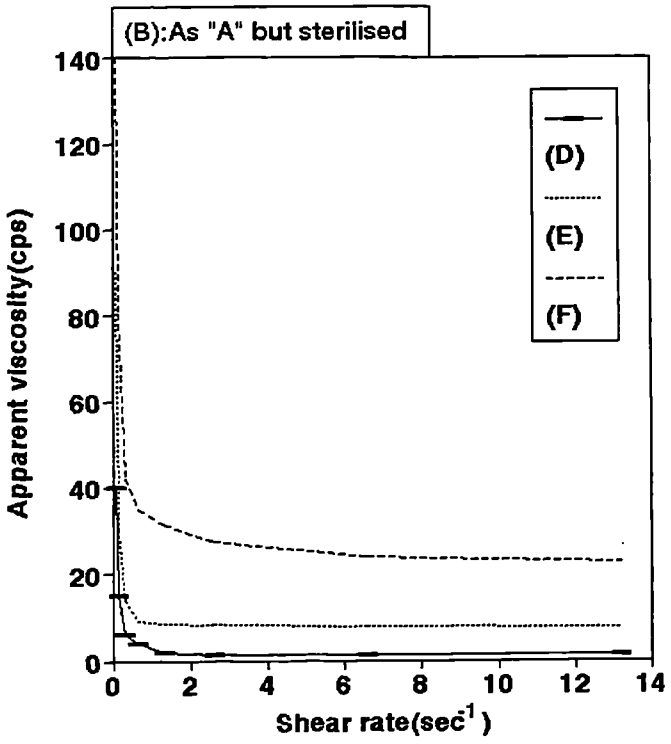
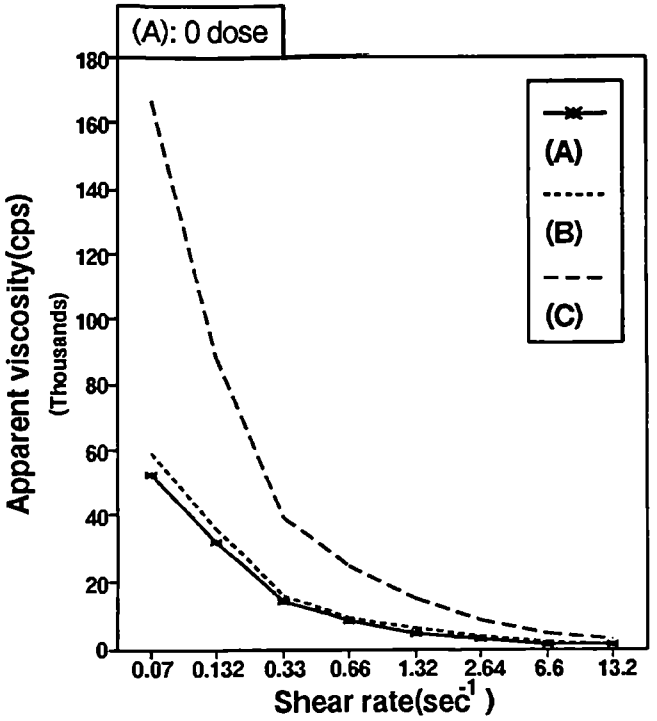
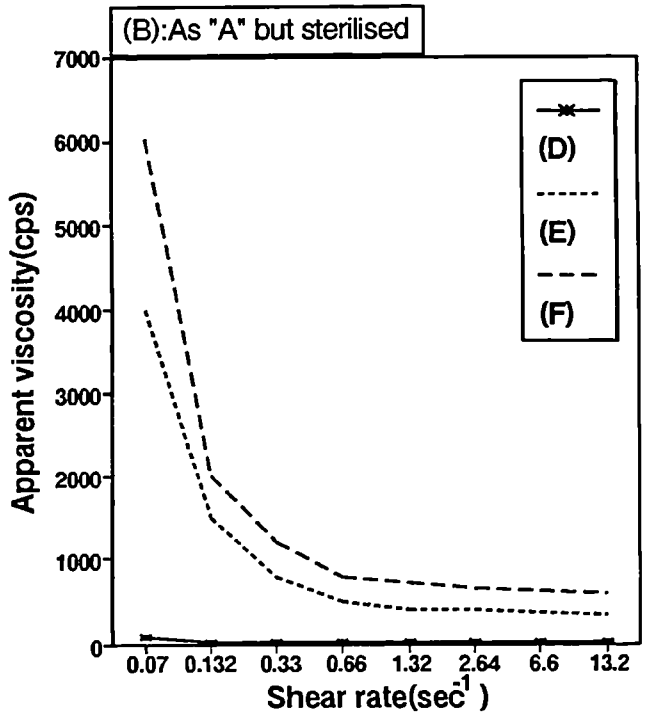


Figure 71: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated up to sterilized dose (25 kGy) of air saturated, (A) LBG (1%) + xanthan gum (1%), (B), LBG (1%) + mannitol (20%) and xanthan gum (1%) and (C) LBG (1%) + mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}) and xanthan gum (1%), (D) as (A) but sterilized, (E) as (B) but sterilized and (F) as (C) but sterilized. Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.



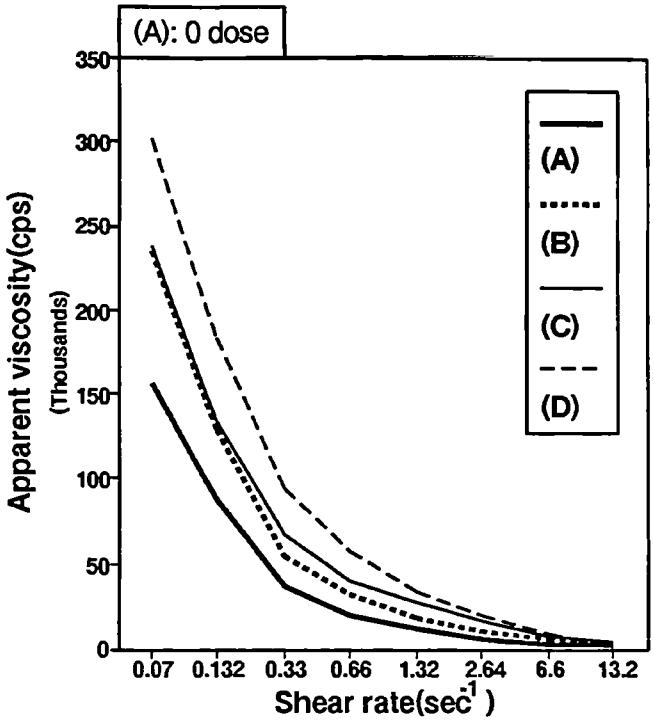


Further experiments were carried out by mixing LBG solution containing mannitol with xanthan gum containing mannitol. The LBG + mannitol and ascorbic acid were combined with the solution of xanthan gum containing mannitol. Further solutions were prepared by mixing LBG containing mannitol with xanthan gum containing mannitol and ascorbic acid and finally by mixing LBG and xanthan gum both containing mannitol and ascorbic acid. The apparent viscosity of these solutions before and after irradiation to a dose of 25 kGy were again measured over the range of shear rate. The results showed a very high viscosity and some stabilisation of the irradiated solutions (Figure 72), though again a decrease in apparent viscosity occurs with increasing shear rates as was previously observed indicating that irradiated solutions are also pseudoplastic. Figure 73 shows the apparent viscosity vs. irradiation dose data for xanthan gum solutions with or without mannitol or NaCl, irradiated in air, N_2, N_2O and N_2O/t-butanol. No substantial differences are observed for irradiation of those solutions irradiated in air and N_2. The greatest decrease in viscosity is found for those solutions irradiated in presence of N_2O. Solutions irradiated in N_2O/t-butanol showed a smaller decrease of viscosity than that found for air, N_2 and N_2O saturated solutions, though for all solutions there is a significant decrease in their apparent viscosity with increasing radiation dose.

Carboxymethyl Cellulose (CMC):

The apparent viscosity of solutions of carboxymethyl cellulose (CMC), CMC, containing mannitol (20%) and CMC, mannitol/ascorbic acid (10^{-2} mol dm^{-3}), were also measured. These solutions were irradiated in air to a dose of 27 kGy and their apparent viscosity measured over a range of shear rates (Figure 74). CMC containing mannitol/ascorbic acid has the highest initial viscosity though after a dose of 27 kGy its viscosity decreases from 177,000 cps to 8,020 cps, a 95% reduction, at a shear rate of 0.07 (0.3 rpm), and from

Figure 72: Apparent viscosity (cps) vs shear rate (sec^{-1}) for unirradiated/irradiated up to sterilized dose (25 kGy) of air saturated, (A) LBG (1%) + mannitol (20%) + xanthan gum (1%) and mannitol (20%), (B) LBG (1%) + mannitol (20%) + ascorbic acid (10^{-2} mol dm^{-3}) and xanthan gum (1%) + mannitol (20%), (C) LBG (1%) + mannitol (20%) + xanthan gum (1%) + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) and (D) LBG (1%) + mannitol (20%) + ascorbic acid (10^{-2} mol dm^{-3}) and xanthan gum (1%) + mannitol (20%) + ascorbic acid (10^{-2} mol dm^{-3}). Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.



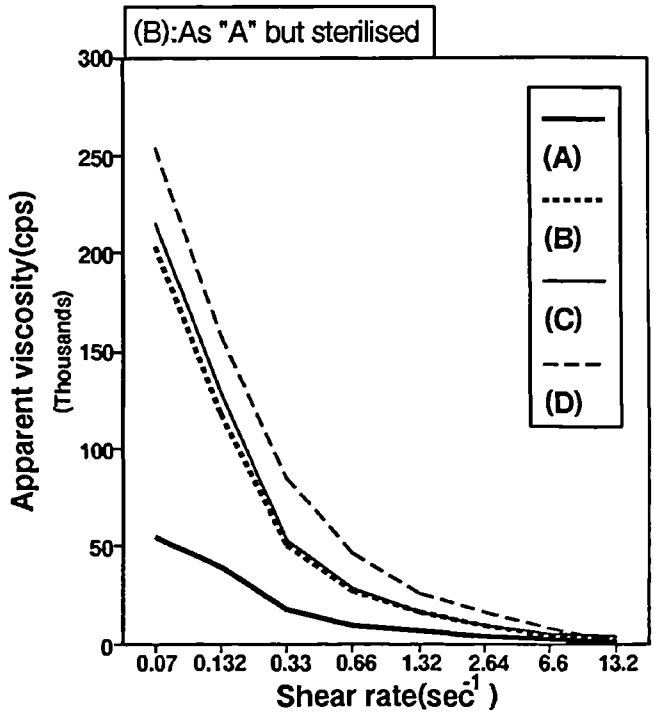


Figure 73: Effect of γ-irradiation on the aparent viscosity of air, N_2, N_2O and N_2O/t-butanol saturated, (A) xanthan gum (1%), (B) xanthan gum (1%) + NaCl (0.1%), (C) xanthan gum (1%) + mannitol (20%), (D) xanthan gum (1%) + mannitol (20%) and NaCl (0.1%). Using a Brookfield L.V.T. viscometer at 60 rpm and spindle number 18, T = 20°.

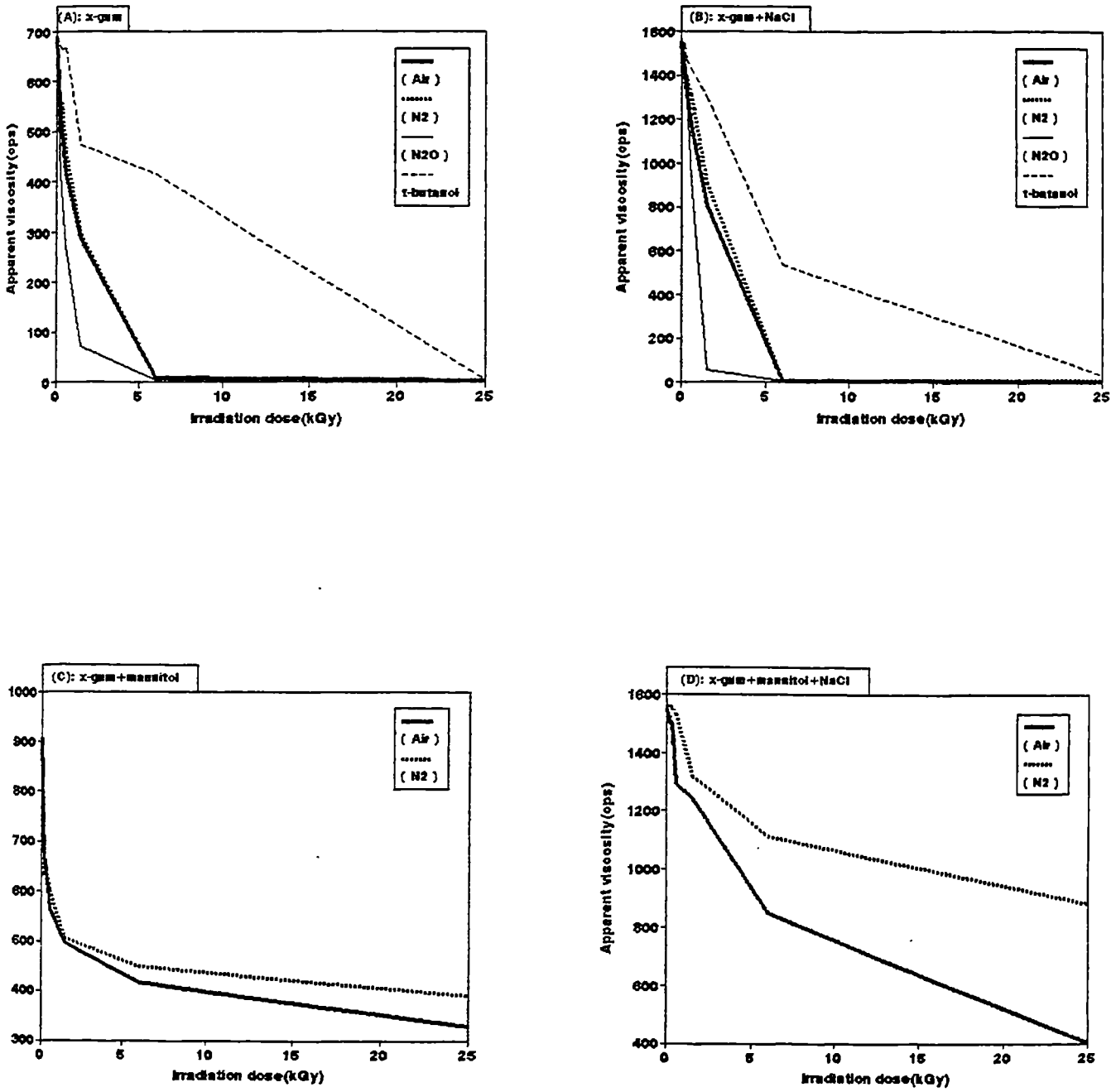
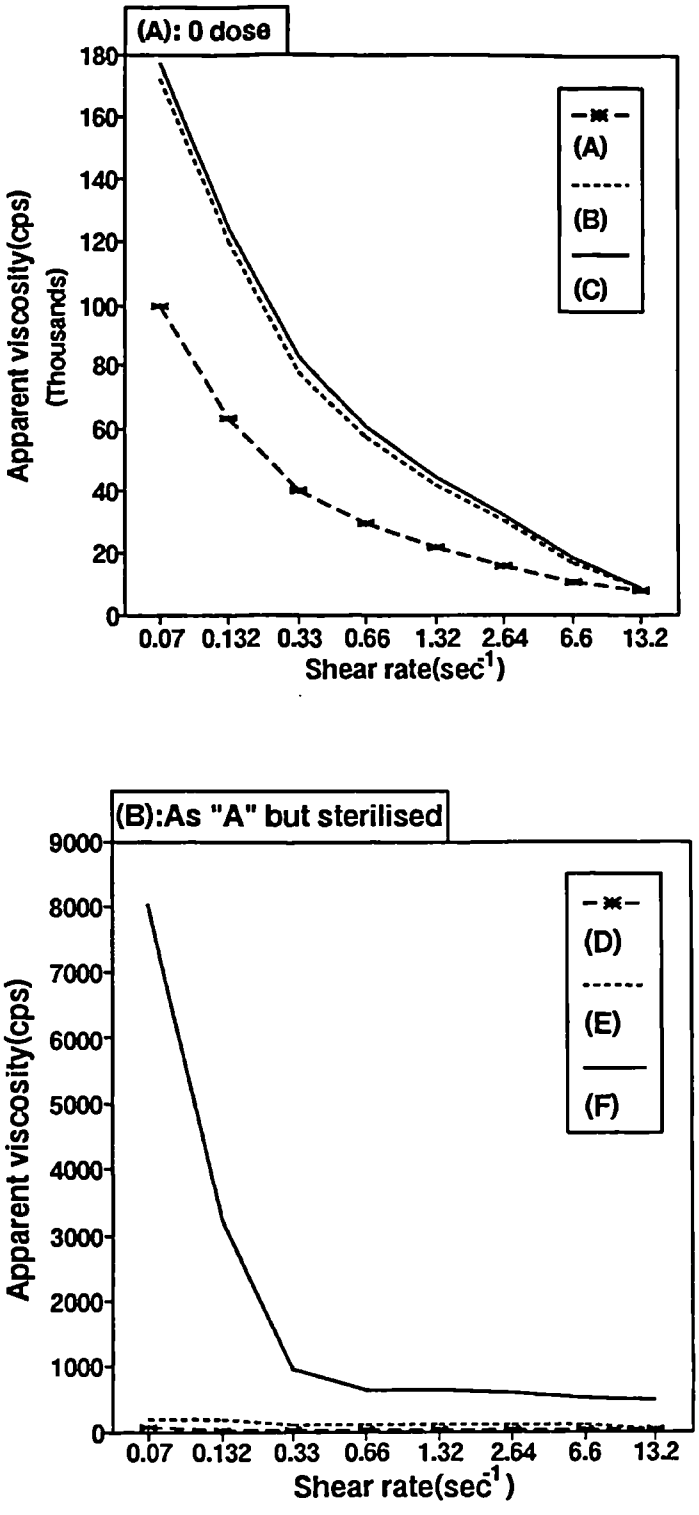


Figure 74: Apparent viscosity (cps) vs shear rate (sec^{-1}) of solutions A [carboxymethyl cellulose (CMC)], B [CMC +mannitol (20%)], C [CMC + mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3})], D [A, irradiated to 27 kGy], E [B, irradiated to 27 kGy] and F [C, irradiated to 27 kGy]. Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.



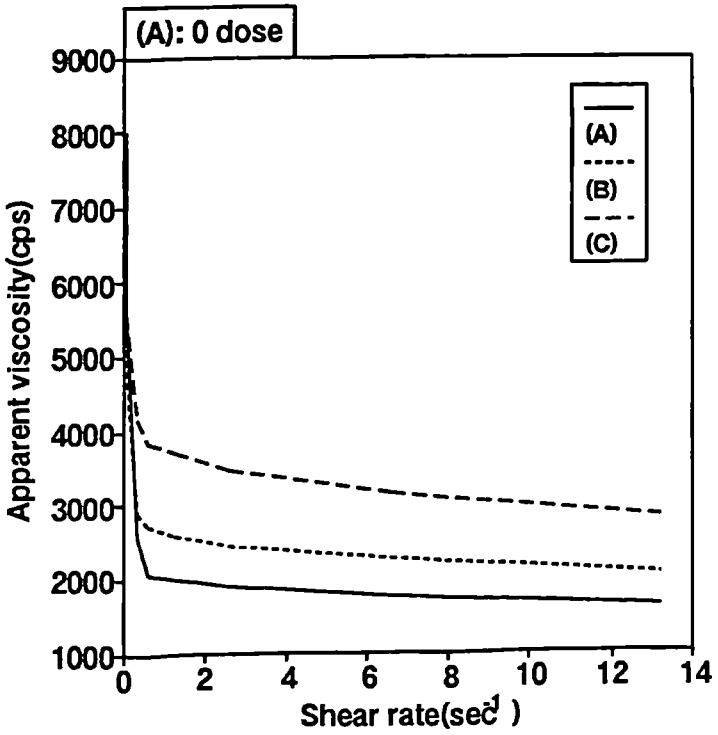
8,950 cps to 481 cps (again ~ 95% reduction) at shear rate of 13.2 (60 rpm). The decrease in apparent viscosity with increasing shear rates, again indicating that CMC solutions are pseudoplastic.

The apparent viscosity of manugel DMB, manugel DMB containing mannitol (20%) and manugel DMB, mannitol/ ascorbic acid were measured to provide similar apparent viscosity measurement conditions as for other gums studied (xanthan gum, LBG, CMC). Solutions were irradiated in air to a dose of 27 kGy (Figure 75B and their apparent viscosities measured as were the viscosities of the unirradiated solutions (Figure 75A). Addition of mannitol (20%) and mannitol/ascorbic acid to manugel DMB solutions (2%) result in an increase in the apparent viscosity (Figure 75).

The apparent viscosity of all of the gum solutions, manugel DMB (2%), xanthan gum (1%), LBG (1%) and CMC were examined in order to compare their viscosities at the same time with the same viscometer and same spindle number over a range of shear rates (Figure 76). All these solutions were also irradiated to 27 kGy (Figure 76B)

Mannitol (20%) was also included in the above four solutions (Figure 77). The results were in similar order to the data in Figure 76, except that there was an increase in the apparent viscosity for all irradiated solutions, indicating some stabilisation of the solution occurs in the presence of mannitol, though the extent of viscosity after a dose of 27 kGy is greater for xanthan gum solutions than for other gum solutions. Addition of mannitol/ascorbic acid (10^{-2} mol dm^{-3}) to the above solutions results in an increase in the apparent viscosity at all solutions (Figure 78). These solutions were irradiated to 27 kGy and their apparent viscosity was again measured over the range of shear rates. CMC again has a highest initial viscosity

Figure 75: Apparent viscosity (cps) vs shear rate (sec^{-1}) of solutions of A [manugel DMB (2%)], B [DMB + mannitol (20%)], C [DMB + mannitol and ascorbic acid (10^{-2} mol dm^{-3})], D (A, irradiated to 27 kGy), E (B, irradiated to 27 kGy), F (C, irradiated to 27kGy). Using a Brookfield L.V.T. viscometer and the appropriate spindle, T = 20°.



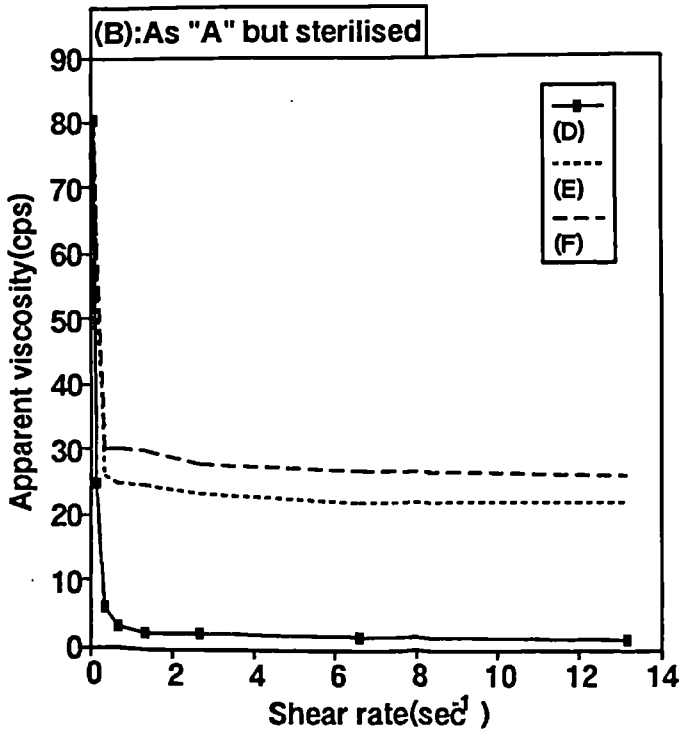
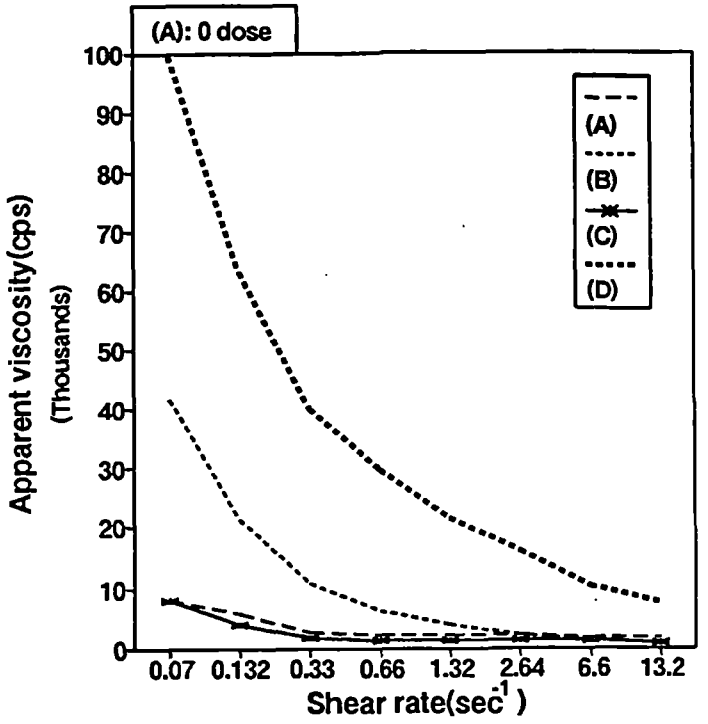


Figure 76: Comparison measurements of apparent viscosity as a function of shear rate for air saturated solutions of A (manugel DMB) 2%, B (xanthan gum)1%, C [locust bean gum (LBG)] 1%, D [carboxymethyl cellulose (CMC)], E (A, irradiated to 27 kGy), F (B, irradiated to 27kGy), G (C, irradiated to 27kGy) and H (D, irradiated to 27kGy). Using a Brookfield L.V.T. viscometer, spindle number 25, (A,B,C,D) and spindle number 18 (E,F,G,H). T = 20°.



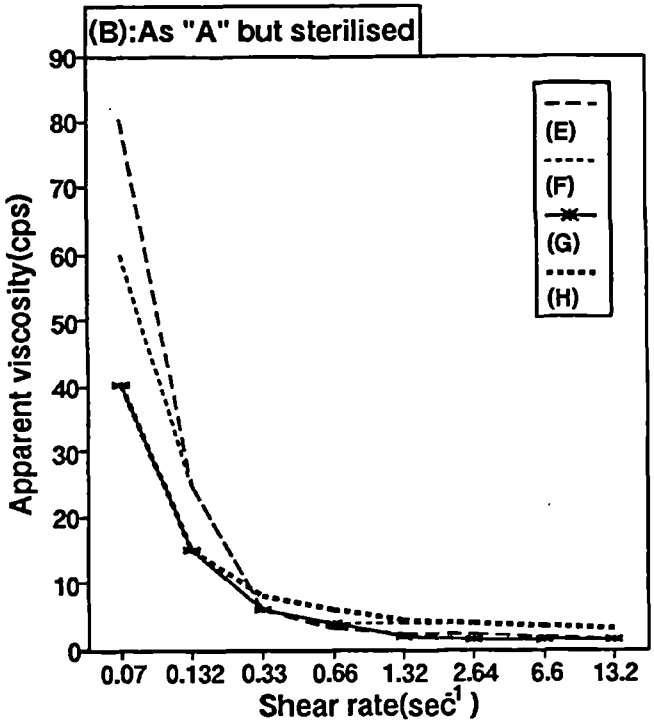
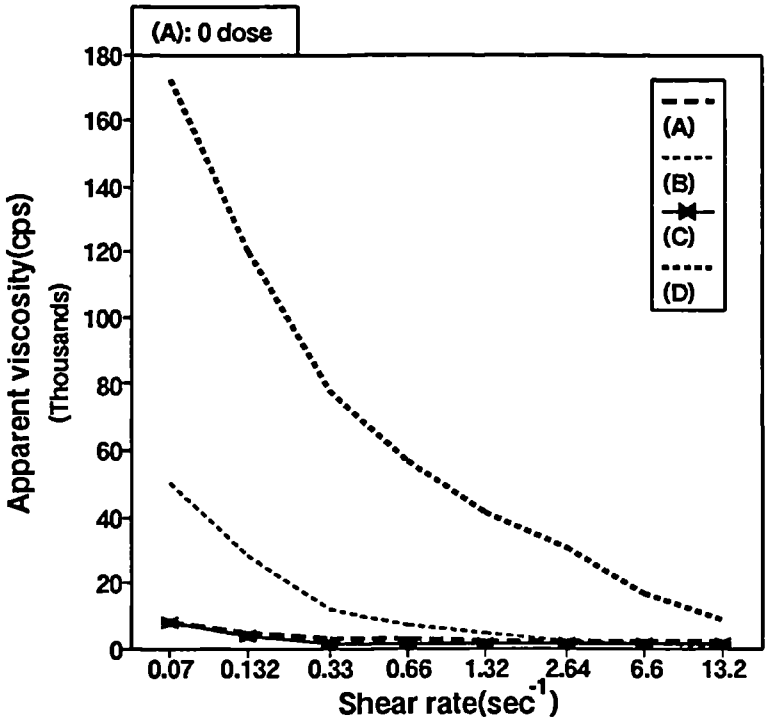


Figure 77: Comparison measurements of apparent viscosity as a function of shear rate for air saturated solutions of A (manugel DMB) 2%, B (xanthan gum)1%, C (LBG) 1% and D, (CMC), all containing mannitol (20%), E (A, irradiated to 27 kGy), F(B, irradiated to 27 kGy, G(C, irradiated to 27 kGy) and H (D, irradiated to 27 kGy). Using a Brookfield L.V.T. viscometer and approprate spindle, T = 20°.



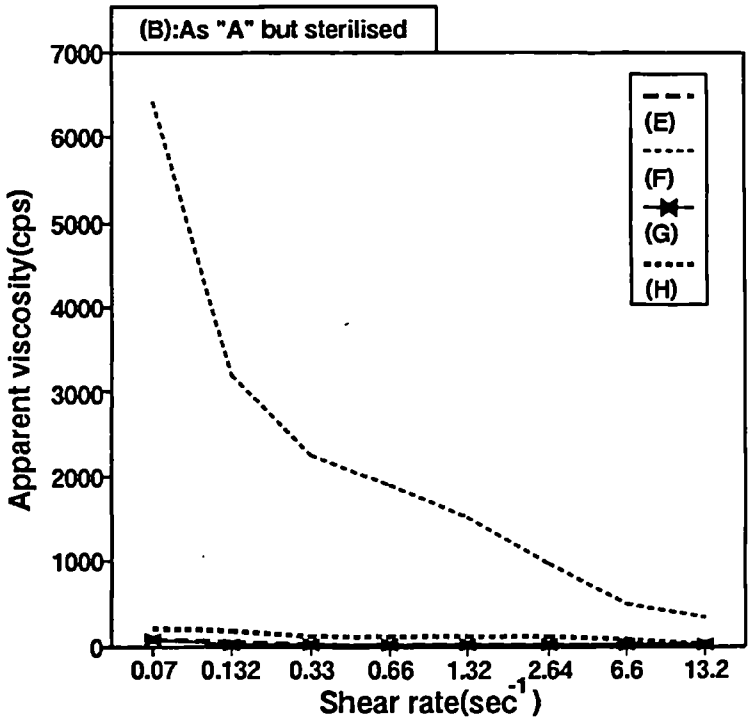
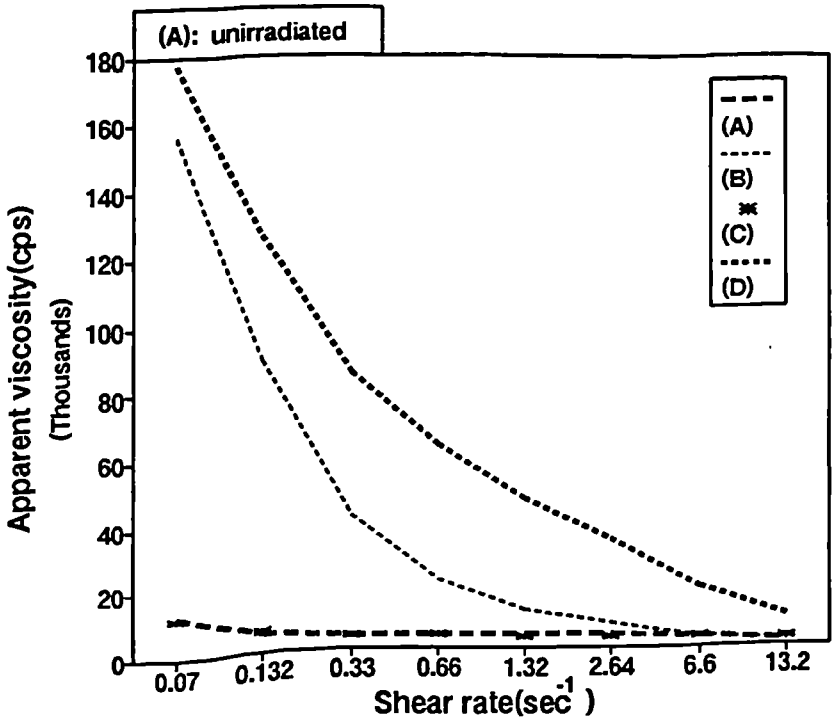
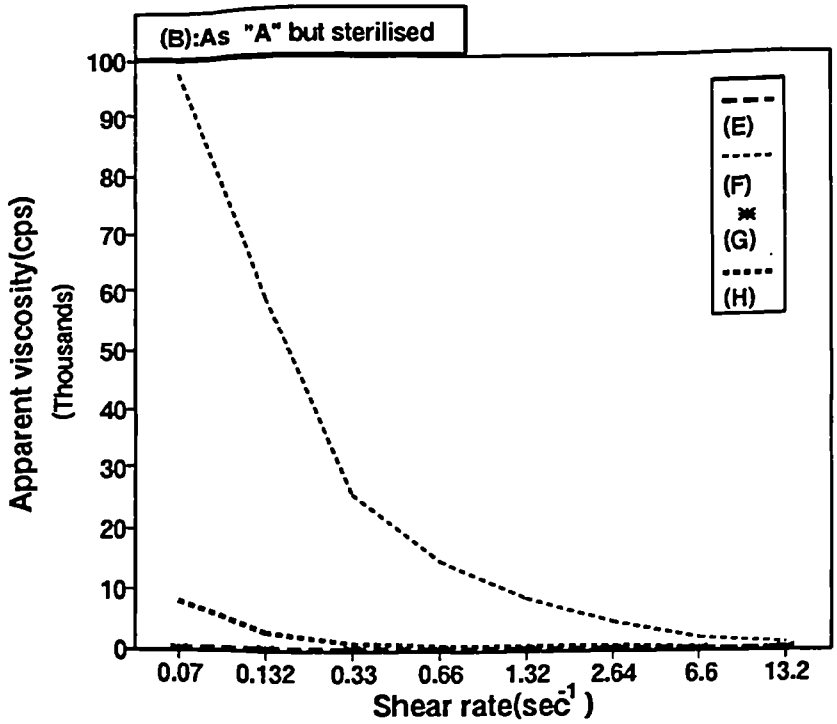


Figure 78: Comparison measurements of apparent viscosity as a function of shear rate for air saturated solutions of A (manugel DMB) 2%, B (xanthan gum)1%, C (LBG) 1% and D, (CMC), all containing mannitol (20%)/ascorbic acid (10^{-2} mol dm^{-3}), E (A, irradiated to 27 kGy), F(B, irradiated to 27 kGy, G(C, irradiated to 27 kGy) and H (D, irradiated to 27 kGy). Using a Brookfield L.V.T. viscometer and approprate spindle, T = 20°.





though after irradiation to 27 kGy, all solutions are extensively degraded and the viscosities dropped to less than 100 cps.

The viscosity of irradiated xanthan gum is again higher than other gum solutions. However, a similar decrease in apparent viscosity occurs with increasing shear rates (spindle speed) as was previously observed again indicating that all of these gum solutions alone, and including mannitol or mannitol/ascorbic acid are pseudoplastic.

CHAPTER FIVE

DISCUSSION

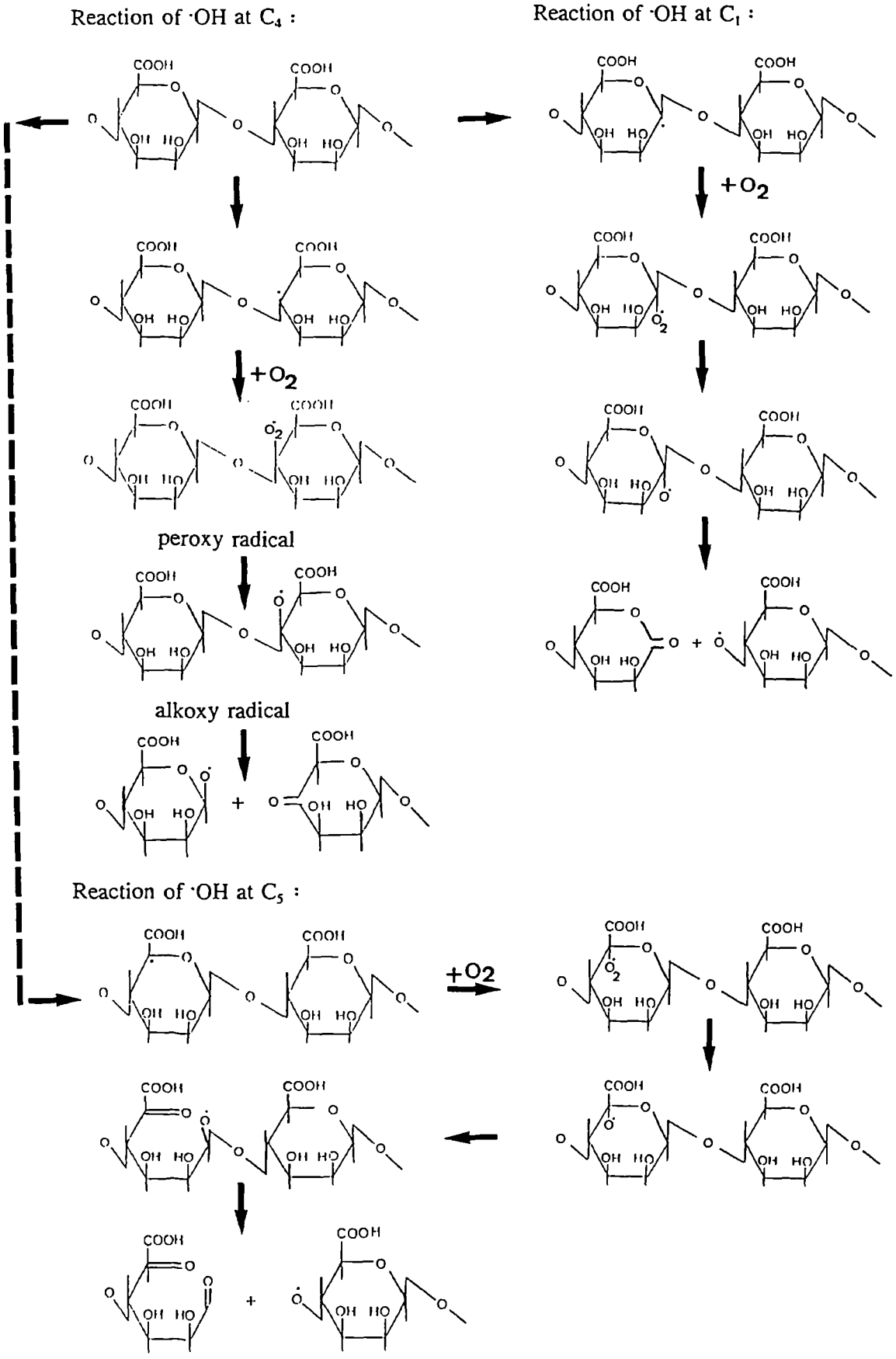
DISCUSSION

Alginates

The alginates used were manugel DMB, manugel DPB and manucol DMF. They contain different ratios of manuronic acid/guluronic acid (M/G) and this ratio effects their gelling properties. Alginates with a low G-block content produce gels which can be considerably deformed before breaking (ie. are more "elastic") compared with gels produced from high G-block alginates. However aqueous solutions of both high and low G-block alginates at concentrations of $\geq 1\%$ are non-Newtonian, ie. pseudoplastic. As the shear rate increases the apparent viscosity decreases. This is known as "shear thinning". The viscosity is also dependent on temperature, the degree of polymerisation and the presence of other substances present in solution (eg. metal ions Na^+, Ca^{2+}) [368].

The data shown in Figure 17 confirms that 2% solutions of the sodium salt of the three alginates used here are all pseudoplastic up to a spindle speed of 60rpm though the viscosity for manugel DPB (which has a high G-block) is considerably greater than for the other preparations over the entire range of spindle speed used. No information is available of the value of the molecular weights of the samples, though the particle size of DPB is greater than that of manugel DMB and manucol DMF[387]. Addition of 15% mannitol causes a small increase in viscosity but does not effect the rheology of the solutions. There is a rapid decrease in viscosity of solutions irradiated up to a dose of 0.5 kGy and the initial rate of viscosity decrease is unaffected by the presence or absence of air (Figures 19-21). Such data was also observed by others[369, 371]. Moreover, the solutions are pseudoplastic up to a dose of 1kGy (Table XVIII). The decrease in viscosity is due to chain breaking as a result of reaction of ·OH radicals with the polymers. The mechanism for this process may be as in Figure 79. These mechanisms explain the well established data that following irradiation

Figure 79: Possible mechanisms for the depolymerization of alginate solutions following irradiation in air.



solutions of polysaccharides lose viscosity, produce an increase in the number of reducing end groups (aldehydes, ketones)[179-185].

In the presence of 15% mannitol, the initial rate of decrease in apparent viscosity of irradiated alginate solutions is less than that observed when mannitol is absent (Figure 22-24), so that the apparent viscosity of all the solutions is $\geq$500cps after a dose of 3kGy for solutions irradiated in air and N_2. This protective effect can be explained by the scavenging by mannitol, at least in part, of ·OH radicals.

The molecular weight of the repeating uronic acid (sodium salt) in the alginate is 198 and 2% solutions (used here) have a molarity of uronic acid moieties of 0.101 mol dm^{-3}, whereas the concentration of mannitol is 0.824 mol dm^{-3}. The bimolecular rate constant for reaction ·OH + mannitol is 1.5 x 10^9 mol^{-1} dm^3 s^{-1} [372]. The corresponding value for alginate is 2.7 x 10^8 mol^{-1} dm^3 s^{-1} and is typical of the value found for other anionic polysaccharides[388], (hyaluronic acid, chondroitin sulphates, N-desulphated heparin). On the basis of the above data it is estimated that only ~2% of the ·OH radicals react with alginate which would result, therefore in almost complete protection of the polysaccharide, hence reducing the extent of depolymerization and solutions should retain their viscosities, at least at low doses.

The major products of mannitol irradiation are mannose and fructose,[144,145,370], and sugars such as these have also been used as ·OH scavengers[199]. The radiation protection effect of mannitol should therefore be prolonged even to substantially higher doses. The lack of complete protection by mannitol suggests that mannitol radicals react with the alginates. A recent paper indicated that protein A-sepharose columns can be only partially protected by mannitol and it was suggested that the mannitol radicals are reactive in this system[371].

Furthermore Sakhri has shown that scission of the glycosidic bond in irradiated 4-nitrophenyl β-D-glucophyranoside solutions occurs in the presence of mannitol, even at high concentration of mannitol which would be expected to afford complete protection to the sugar[373].

Pre-sterilisation of Alginate Solutions and Thickening Agents:

The most effective method used to thicken alginate solutions was that using calcium orthophosphate and δ-gluconolactone (method 3). Unirradiated 2% alginate solutions (DMB, DPB, DMF) could be thickened and produced gels, the viscosity of which were too high to be measured (Table XXIV-XXVI). But irradiated alginate solutions (25kGy) did not form gels, due to degradation of the polymer structure. Gels could be formed when mannitol was included in the irradiated alginate solutions (25kGy) again indicating the protective effect of mannitol.

When both alginate and mannitol solutions and the gelling agent solutions were irradiated separately and then mixed, gels were formed indicating that pre-sterilization of the components by irradiation is a feasible method of preparation of sterile gels.

Radiolysis of Alginate Gels on a Nylon-Mesh Support:

Gels of this type have potential use of wound care[299-302, 374-376]. Those prepared here could bend easily without cracking, but bacterial growth occurred when stored at room temperature. Irradiated to 25kGy, the gels crack easily by becoming more brittle, are easily squashed and lose water. Inclusion of mannitol improves the quality of the gel and again indicates its protective role in the systems. No such observation for similar gels have been previously reported. No further beneficial effects were found when ascorbate (0.5%) was

also included in the gel.

Previous studies on irradiated sepharose-protein A columns[371], indicated that enhanced protection occurred when ascorbate was included with mannitol as radical scavengers and it was suggested that mannitol radicals are repaired by ascorbate. Other studies have indicated that ascorbate can protect systems by directly scavenging ·OH radicals[377]. Gels containing a higher ascorbic acid concentration (5%) were of poor quality and could not be used for the purpose of wound dressing. The lack of further protection by ascorbate may be due to its low concentration in the gel. The lack of protection of alginate by ascorbic acid was also observed by Redpath and Willson[378].

A second important property of alginate gels is their ability to take up aqueous fluids. Unirradiated gels (with or without mannitol and ascorbate) take up varying amounts of water and saline, DMB taking up the most and is easily manipulated. After irradiation to sterilizing doses, DMB gels have some capability to take up water, but when mannitol is included, the performance of the irradiated gel is considerably improved showing the protective effect of mannitol. The presence of ascorbate as well as mannitol decreases the performance of the gel, the gel being worse than alginate (DMB) alone, suggesting that ascorbate decreased gel stability, possibly by binding some calcium responsible for gel formation. DPB and DMF gels are less stable to radiation. DMB is also more stable in the presence of saline than DPB or DMF. Gel capability to take up saline decreases on irradiation. Moreover, after exposure to saline for ~50 minutes the amount of saline uptake decreases, presumably due to sodium displacing some calcium ions responsible for maintaining gel structure. The same pattern of protection by mannitol was again observed as previously found for water uptake.

Gels that were concentrated by water evaporation were more stable to irradiation (25kGy). Gels without mannitol, concentrated by a factor x2, x3 and x4 increase their capability to take up water and saline (Figure 32A). The stability of the irradiated gels (24kGy) is indicated by their ability to take up 80% and ~25% of their weight of water and saline respectively. This may be because in the concentrated gels there is a greater extent of energy absorption by the alginate itself, the system more resembling that found in the solid state. In general, carbohydrates are more stable to radiation in the solid state than in aqueous solutions[199,200]. After immersion in saline of the unirradiated, x4 concentrated gel for an extended period (72hrs), the gel remained intact, whereas the irradiated gel remained intact, but took up less saline than the unirradiated gel.

Drying of gels containing mannitol (initially 15%) causes them to go opaque, due to some precipitation of mannitol. Immersion of these gels in water and saline caused an initial decrease in gel weight, presumably due to washing out of the precipitated mannitol (Figure 33A and 35A), though those gels take up water and saline. The gels that originally contained mannitol now appear to be less stable to irradiation (Figure 33D and 35D) and resemble those prepared without mannitol. The process of gel concentration appears to cause removal of some of the mannitol from the gel and less protection is afforded to the alginate gel.

Another parameter associated with gel destruction by irradiation is water release (measured by decrease in gel weight). The decrease in weight of 2% gels is greater by a factor of ~3 for gels not containing mannitol, a further indication of its protective effect. Dried gels lose relatively little water and those containing mannitol lose none at all (Figure 36 and Table XXX).

The 2% gels, irradiated to 16kGy cannot be bent, though dried gels can be bent through an angle of up to 100° before they rupture (Figure 37A). Gels containing mannitol were more stable and dried gels irradiated to 24kGy and immersed in water for 60 minutes can bend through 180° (Figure 37D).

The gels that had the greatest capability to take up saline and to be manipulated most easily (both before and after irradiation) were those that contained initially 2% alginate and 5% mannitol and dried to a quarter of their original weight (ie. the gel now contained 8% alginate and 20% mannitol). These gels were clear, pliable and, after irradiation to 30kGy, remain stable in saline for up to 24 hours.

This study has confirmed the previous observations that irradiation cannot be used to sterilize alginate solutions[379]. Autoclaving and dry heat are also not suitable, causing decreased viscosity[380]. Ultra-filtration (0.45μ pore) is effective but is very slow, $<50cm^3$/hour [380]. Ethylene oxide is also effective[380], but its use is now questioned because of doubts over its safety for preparations to be used in the food and pharmaceutical industry[381].

The results of this study suggests that high viscosity sterile alginate solutions can be obtained by mixing previously sterilized solutions of alginate containing mannitol with the sterilized thickening agents δ-gluconolactone and calcium orthophosphate. Such solutions may be of use in the pharmaceutical industry.

Textile fibres produced from alginates (sorbsan) are now commonly used for wound care. This study suggests that wet alginate gels may also be of some benefit in wound care. They may be prepared including mannitol which renders them relatively stable after sterilization

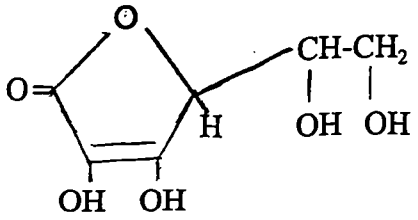
by irradiation. They are smooth, soothing to the skin, absorb saline, are easily removed, are sufficiently flexible to adapt to the size and geometry of the wound or cavity and the possibility exists for incorporating other drugs into the gel.

Xanthan Gum:

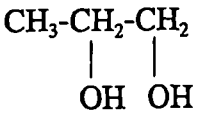
Xanthan solutions are susceptible to bacterial action (optimum temperature 35-40°C)[382], and require a preservative to ensure their long term stability[383]. The objective of this study is to produce xanthan solutions of high viscosity that had been irradiated to doses required for sterilization. The initial studies indicated that all the solutions were pseudoplastic (ie. the apparent viscosity decreases with increasing shear rate). A 3-4 fold increase in apparent viscosity was found when NaCl and LBG were added to xanthan and similar data were observed when mannitol and ascorbic acid were also included (Figure 42,43). The highest initial viscosity of ~275,000 cps was measured for a solution containing xanthan (1%) mannitol (20%), ascorbic acid (10^{-2} mol dm^{-3}, 0.18%), NaCl (0.1%) and LBG (1%), (Figure 44).

Addition of ascorbic acid to LBG/xanthan/NaCl/mannitol increased the viscosity by ~ 60%, an increase similar to that found for some organic acids with xanthan alone[338].

Some plasticisers (eg. propylene glycol) and water-soluble resins also increase the viscosity of xanthan[338], and it is possible that the structural similarity between ascorbic acid and some glycols may account for these data.



Ascorbic acid



Propylene glycol

The order of mixing the solutions had no effect on their final overall viscosity and no changes in viscosity were observed for solutions saturated with air, N_2, N_2O or N_2O/t-butanol (1 mol dm^{-3}). Irradiation of xanthan solutions, in the absence and presence of thickeners (NaCl, LBG) caused a rapid initial decrease in viscosity in all instances and after a dose of 25kGy the solutions are pseudoplastic and have apparent viscosity of ~200-400 cps. t-Butanol had some protective effect on xanthan-LBG and xanthan-NaCl solutions. Parsons, *et al*[384], have attributed the depolymerization of xanthan solutions to reaction of ·OH radicals with the polymers. Glycosidic bond scission in cellobiose[385], glycosaminoglycans[179-185], and aryl glycosides[199,200,389], initiated by ·OH radicals is well established. Addition of mannitol to xanthan only partially protects the solutions and suggests that mannitol radicals produced by reaction of ·OH with mannitol, may be reacting with the xanthan. A similar observation was made by Moore *et al*[371] who found that radicals derived from mannitol and sorbitol did not effectively protect protein A-sepharose columns irradiated to high doses (25 kGy). When NaCl was also present the solutions could still be thickened and have a viscosity of ~900cps after irradiation to ~25 kGy, (Figure 73D). Even greater thickening was possible when LBG and ascorbic acid were also present in xanthan solutions irradiated to 25 kGy. The length of the xanthan polymer fragments must be sufficiently large to allow the thickening process to proceed and viscosities up to 3500 cps could be achieved. The solutions with highest apparent viscosity were those prepared by mixing equal volumes of solutions of LBG (1%), mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}) and solution of xanthan gum (1%),

mannitol (20%) and ascorbic acid (10^{-2} mol dm^{-3}). These solutions contain potential radiation protections and thickeners, since radicals derived from mannitol react with ascorbate (k_2 $\sim 3.5 \times 10^7$ mol^{-1} dm^3 s^{-1})[386], and as previously described, LBG is widely used to thicken xanthan. The viscosity of this solution falls from 300,000 cps to ~250,000 cps (shear rate 0.07 s^{-1}) after irradiation to 25 kGy. The solution was extremely thick almost on the point of forming a gel.

The concentration of primary radicals $\bar{e}_{aq}$, H· and ·OH produced by 10Gy is ~6μmol dm^{-3} based on $G\dot{O}H = G\bar{e}_{aq} = 2.8$, $GH^{\cdot} = 0.6$ (per 100 ev). The sterilization dose of 25kGy therefore yields ~15m mol dm^{-3} radicals which is ~70 times less than the mannitol concentration. Mannitol would not be degraded by more than ~1.5% under these conditions. The 0_2 concentration in air-saturated water is 0.26m mol dm^{-3} which is ~ 1/60 that of the total radical yield at a dose of 25 kGy. 0_2 is therefore rapidly consumed and after a dose of ~0.5 kGy 0_2 should be removed (assuming peroxy radicals of the type $R\text{-}0\dot{-}_2$ do not yield oxygen). This, however is not the case since such radicals can disproportionate to yield 0_2. However, at the high dose used, the role of 0_2 decreases and the solution will ultimately become anaerobic. Previous studies on sepharose-protein A columns indicated that this system becomes anaerobic after a dose of 2.98 kGy (~1/8) the dose generally accepted for the purpose of sterilization[371].

Whereas xanthan solutions are readily depolymerized, by irradiation, a system has been devised which contains a thickening agent (LBG) and radiation protectors (mannitol and ascorbic acid) which can be irradiated to the dose required for sterilization, 25kGy having a post-irradiation viscosity of ~250,000 cps. Such a solution may be of benefit in the pharmaceutical industry for the purpose of preparing sterile, viscous solutions and gels.

Carboxymethyl Cellulose (CMC):

3.5% solutions of CMC are pseudoplastic, (Figure 74), and their apparent viscosity was almost doubled by addition of mannitol (20%), though further inclusion of ascorbic acid (10^{-2} mol dm^{-3}) did not increase the viscosity. Irradiation to 25kGy resulted in a decrease in the apparent viscosity of CMC/mannitol/ascorbic acid from ~180,000 cps to 8,000cps, whereas for solutions of CMC alone and CMC/mannitol the viscosity was less thana 500 cps. This further illustrates the protective effect of ascorbic acid as was observed for xanthan solutions, and also indicates that mannitol radicals cause depolymerization of CMC. However alginate (manugel DMB) was not protected either by mannitol or mannitol/ascorbic acid either in solution or in gel form.

Xanthan gum (1%) and CMC (3.5%) have very high initial viscosities compared with manugel (2%), (40,000, 100,000 and 8,000 respectively). In the absence of scavengers, polysaccharides are depolymerized due to reaction of ·OH radicals at several positions in the molecules. The rate constants for the reaction are all ~10^8 mol^{-1} dm^3 s^{-1}. In no instance did mannitol totally protect the polysaccharides examined in this study, in spite of it being present at very high concentrations where ~90-95% protection would be expected. Even though ascorbic acid enhanced the protective effects of mannitol (due to its reaction with mannitol radicals as previously discussed), some reaction of mannitol radicals with the polysaccharide must still occur and cause depolymerization. No data is available regarding the values of the bimolecular rate constants for reaction of mannitol radicals with the polysaccharides but it would be surprising if significant differences in the values were to occur. Thus it would be expected that the extent of degradation of all the polysaccharides would be similar, but the final viscosity of the alginate would be lower since its initial viscosity is significantly the lower, even though it was initially of similar concentration to

xanthan gum.

Comparison of the data in Figures 74, 75 and 78 indicates that solutions of xanthan, CMC and alginate, containing mannitol/ascorbic acid, irradiated to 27kGy have apparent final viscosities of, 100,000, 8,000 and 80 cps respectively. However, viscous sterile alginate solutions can be obtained if the thickening agents δ-gluconolactone and calcium orthophosphate are also included. All may have uses in pharmaceutical and industrial applications where a viscous sterile preparation is required.

Future Work:

Sterile solutions may be prepared by pre-sterilization of the solid powders, water and the containers, but final preparation would have to be carried out under a sterile environment (eg. sterile hood).

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