# The gamma irradiation of tragacanth: effect on microbial contamination and rheology

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Increasing awareness for the need for pharmaceutical products and their constituents to be free of contaminating microorganisms prompted the present study into the effect of high energy ionizing radiation on tragacanth, the dried gummy exudation, obtained by incision, from species of *Astragalus*. Its widespread use in many pharmaceutical preparations (see Martindale, The Extra Pharmacopoeia 1977), particularly as a thickening and suspending agent, coupled with the fact that it may be highly contaminated because of its natural origins, its mode of collection and storage, makes it suitable for study. The reported deleterious effect of conventional methods of sterilization on the viscosity of tragacanth mucilage (Farley & Lund 1976) points to the possible application of $\gamma$-radiation for reducing the microbial load of this preparation.

The present study is aimed at determining the effect of different $\gamma$-radiation doses on the rheological profile of tragacanth mucilage, prepared from tragacanth irradiated in the dry-state, and the efficacy of such treatments at reducing its pre-irradiation microbial load.

Tragacanth samples were obtained from Hopkins and Williams Ltd., U.K., and E. Merck, West Germany. These samples have been designated HW and M respectively. Mucilages were prepared according to the methodology of the British Pharmaceutical Codex (B.P.C.) 1973 except that sterilized water was used in place of chloroform water so as not to confound the testing for microbial contamination.

Microbiological growth media used were tryptic soy agar (TSA) and Sabouraud dextrose agar (SDA), both obtained from Difco and prepared according to the manufacturer's recommendations.

Enumeration of microbial contaminants was carried out by direct inoculation of appropriate aliquots of tragacanth mucilage onto the solidified growth media, in Petri dishes, and following overnight incubation at 37 °C (25 °C for SDA plates), the resultant colonies scored. Each colony was taken as indicative of a single organism in the original mucilage. Initial attempts at using a membrane filtration technique proved unsuccessful due to the slow rate of filtration. All manipulations were carried out in a laminar air flow cabinet.

Tests for microbial contamination due to salmonellae and *E. coli* were undertaken as stipulated in the British Pharmacopoeia (B.P.) 1973 and its 1975 Addendum respectively, except that MacConkey agar was used in place of MacConkey broth as the test media for *E. coli*.

Viscosity determinations were made using a Haake Rotovisco RV3 viscometer fitted with an NV (coaxial cylinder) sensor system and connected to a plotting recorder. The viscometer was programmed to give a rate of increasing rotor speed of 200 rev $min^{-2}$ up to a maximum speed of 1000 rev $min^{-1}$ (equivalent to a shear rate of 5410 $s^{-1}$) then decreasing at the same rate to 0. All measurements were at 30 $\pm$ 0·5 °C. Routinely, apparent viscosities were computed at a shear rate of 2705 $s^{-1}$ (500 rev $min^{-1}$ rotor speed), on the upward curve of the rheogram.

$\gamma$-Irradiations were carried out as described earlier (Jacobs & Melumad 1976). Radiation doses were over the range 0·1 to 5·0 Mrads and were checked by periodic dosimetric determinations using a Fricke dosimeter (Fricke & Morse 1927) ($G_{Fe^{3+}} = 15{\cdot}3$ (Spinks & Woods 1976)).

Enumeration of contaminants in the unirradiated tragacanth indicated the presence of approximately $2{\cdot}5 \times 10^4$ and $5 \times 10^2$ aerobes $g^{-1}$ of HW and M tragacanth samples, respectively, on TSA medium, and $1{\cdot}3 \times 10^4$ and $1{\cdot}2 \times 10^2$ organisms $g^{-1}$, respectively, on SDA medium.

Tests both for *E. coli* and salomonellae indicated their absence from both tragacanth samples.

Tragacanth mucilage samples were set aside for 5 days at 4° C for complete solvation to occur. Following this interval, viscosity measurements remained constant for up to 30 days (the maximum period tested). No significant differences were observed between the apparent viscosities of similarly irradiated tragacanth samples from the two batches and hence it was elected to pool together these results.

Typical rheograms for the different radiation treatments are presented in Fig. 1. These curves are indicative of shear rate thinning, typical of pseudoplastic liquids, probably due to alignment of randomly orientated linear macromolecules. The commencement of near linearity is conditional on the radiation dose. The 5 Mrad curve is almost linear characteristic of a Newtonian liquid and possibly indicating a breakdown of a gel structure.

The viscosities calculated from each of these curves at a 2705 $s^{-1}$ shear rate have been plotted as a function of radiation dose (Fig. 2). Each point on this curve is the mean of at least three individual determinations. There was a decrease in viscosity with increasing dose, with a pronounced viscosity change over the initial dose range.

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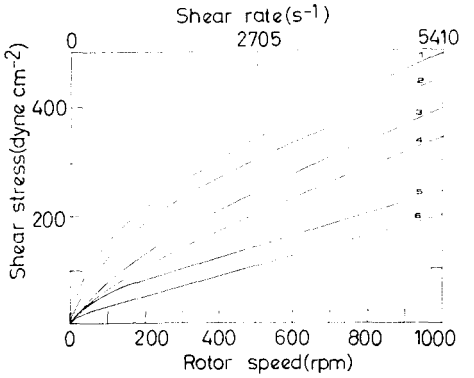


FIG. 1. Typical rheograms of mucilages prepared from irradiated tragacanth. 1–0; 2–0·1; 3–0·5; 4–1; 5–2·5 and 6–5 Mrads.

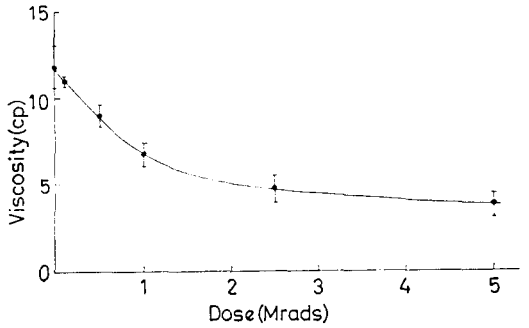


FIG. 2. Effect of radiation dose on the coefficient of viscosity, $\eta$ (s.d.), of mucilages prepared from irradiated tragacanth. Values of $\eta$ have been computed at a shear rate of 2705 $s^{-1}$.

Sterility testing according to the methodology of the B.P. revealed that all irradiated samples were free from contamination, although the 0·1 Mrad irradiation treatment was only carried out on the M (less contaminated) tragacanth sample. The corresponding decrease in viscosity at this minimal radiation dose was about 7%.

Tragacanth consists of a water soluble fraction known as tragacanthin and a water insoluble fraction known as bassorin. The former is composed of units of glucuronic acid and the monosaccharide arabinose joined by glycosidic linkages, whilst the latter is more complex but of a similar constitution (Claus et al 1970).

Whereas many pharmaceuticals, irradiated in the solid state, show remarkable resistance to radiation damage (Diding et al 1978; Holland et al 1967; Jacobs & Melumad 1976; Jacobs 1977a, b; Power 1978), the radiation lability of carbohydrate structures is well documented. For monosaccharides, Phillips (1973) has reported on the extreme sensitivity of D-glucose to $\gamma$-irradiation with the formation of acid and the release of $H_2$. Irradiation of oligosaccharides and polysaccharides results, in addition, in splitting of the glycosidic bonds with the formation, as with cellulose, of stable free radicals (Antoni 1973). These findings, coupled with those of others (Blouin & Arthur 1964; Arthur et al 1965), may help explain the relatively high susceptibility of tragacanth to $\gamma$-rays. Furthermore, the radiation-induced decrease in tragacanth chain length resulting in reduced viscosity of the mucilage may be predicted from the modified Staudinger equation (Houwink 1940),

The marked initial decrease in viscosity with increasing dose is not peculiar to tragacanth alone. Irradiated α-D-glucose also shows a decrease in the rate of breakdown with increasing dose (Phillips 1973).

Although even small radiation doses (<1 Mrad) have a pronounced effect on the viscosity of tragacanth, minimal doses of (say) 0·1 Mrads may be used for reducing its microbial load particularly if the initial contamination is not excessive.

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