Dextran sulfate

Trade name: Dextran Sulfate, high- and low sulfated

Chemical names: Dextran, hydrogen sulfate sodium salt
Dextran sulphate
Sodium dextran sulfate
Dextran polysulfate

Catalogue number: DB004, DB006, DB008, DB012, DB016, DB050, DB054, DB005, DB007, DB009, DB013, DB003, DB015, DB051

CAS nr. 9011-18-1

Structure:

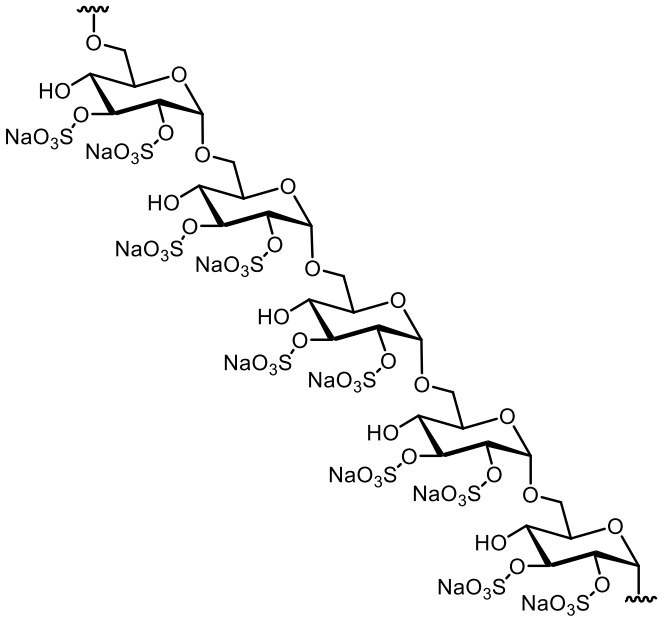

Figure 1. Structural representation of a fragment of a highly structured dextran sulfate. Higley structured dextran sulfate contains approximately two sulfate groups per glucose unit, giving a degree of substitution of 2.0.

Introduction

Dextran sulfates (DS) can be produced with different weight average MW, different MW distributions, different degrees of substitution, different counter ions (e.g. potassium instead of sodium) and by different sulfation procedures. Thus, for example the designation dextran sulfate 70 will not fully identify the compound and this must be taken into account when evaluating the performance of these compounds.

DS are manufactured by treating a selected dextran fraction with a sulfating agent (e.g. chlorosulfonic acid) and thereafter carefully purifying the reaction mixture to obtain the product as a white powder. The sulfate content is controlled by the reaction conditions but for most purposes a sulfur content of 16-20% are acceptable.

Structure and physical-chemical properties

Structure

The structure of a highly sulfated DS as shown in Fig.1 reveals approximately two sulfate groups per glucose unit in the dextran chain. The term degree of substitution is widely used to designate the number of substituents per glucose chain unit. Thus, the above structure would be assigned a degree of substitution of 2.0. The distribution of sulfate groups has been studied by several authors and indicates that at low degree of substitution the 2 and 3 positions dominate. As the degree of substitution increases, the proportions of 2,3 and 2,4 substituted units increases. However, these results are found to be dependent also on the method used for sulfation1. Further studies based on 13C and 1H NMR provide unequivocal evidence that in highly substituted dextran sulfate samples, the substitution at C2 and C3 is almost complete 2,3.

To establish whether all dextran chains in a sample are uniformly substituted requires separation techniques based on charge density. In unpublished studies, a DS with a degree of substitution of 0.37 could be separated into fractions with sulfur contents 0.3 to 14%. However, this may also be due to the method of preparation. At higher degree of substitution, the fractions showed more uniform distribution of sulfate groups (unpublished studies).

Molecular weight

The dimensions of dextran sulfate fractions are best described in terms of Mw, Mn and ratio Mw/ Mn. These values are generally determined by gel permeation chromatography which gives valuable information on the molecular weight distribution. A typical distribution is shown in Fig. 2. It should be noted that the value for the Mw obtained by size exclusion chromatography is a relative value as the column is calibrated with dextran fractions since no standard dextran sulfate fractions are available. The values obtained may vary according to the columns and other parameters used. The Mw obtained by light scattering measurements (LS) is somewhat lower than that obtained by GPC (see Table 1). Values obtained are not greatly dependent on the salt concentration used in the solvent but will be affected by weighing errors, intensity of scattered light, dn/dc and extrapolation errors (unpublished studies).

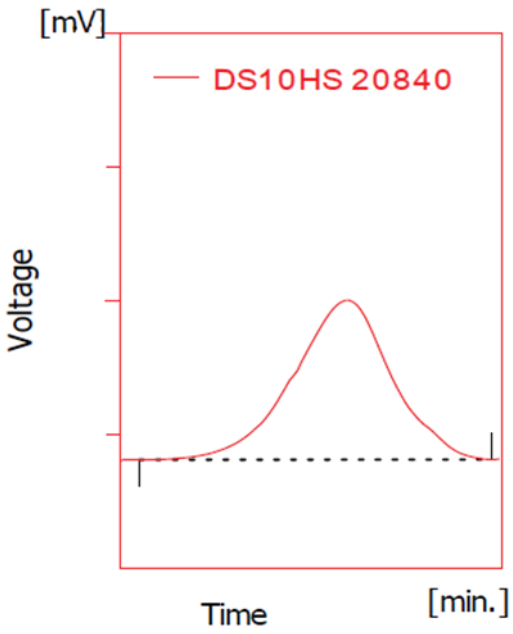

Figure 2. A typical weight distribution of dextran sulfate 10. *The dimensions of dextran sulfate fractions are best described in terms of M_w, M_n and ratio M_w/ M_n. These values are generally determined by gel permeation chromatography which gives information on the molecular weight distribution.*

Batch no.	Degree of substitution	Mw GPC	Mw LS
14D07B	0.82	124 000	115 000
14G32A	1.2	23 100	21 500
14G24B	1.92	12 800	10 400
14G12A	2.1	4 800	3 000

Table1. Comparison of Mw values by GPC and light scattering (LS). *The MW obtained by GPC is relative due to the fact that no standard dextran factions are available. Values may vary according to columns used and other parameters. The Mw obtained by light scattering measurements (LS) are slightly lower than those obtained by GPC.*

Values obtained are not greatly dependent on the salt concentration used in the solvent but will be affected by weighing errors, intensity of scattered light, dn/dc and extrapolation errors (unpublished studies). It is important to note that the distribution can, if not controlled, give variable response in the system to which it is added whether this be a biotech process, diagnostic system or a formulation. It is therefore crucial that all parameters that can affect the distribution, e.g. synthesis or determination are carefully controlled. The calibration should be checked continuously against relevant standards.

Viscosity

Dextran itself is considered to exist in solution as a flexible statistical coil. Introduction of charged sulfate groups along the chain will cause an expansion of the coil due to electrostatic repulsion. This will also

be dependent on the degree of dissociation into polyanions and counter-ions. At high salt concentration (0.5M NaCl) the electrostatic forces are neutralized, and the coil behaves as if it were uncharged. Intrinsic viscosity measurements confer useful information on the molecular size and flexibility. At high degree of substitution, the [η] decreases when the salt concentration is decreased whereas at lower degrees of substitution (0.01 – 0.37), the [η] values tend to show a slight increase. Measurements of the [η] in 0.5M NaCl show little change in the range DS 0.5 – 2.0 (See table 2).

Degree of substitution	0.5M NaCl	0.15M NaCl	0.005M NaCl
Unsub.	0.270	0.264	0.258
0.02	0.250	0.263	0.281
0.23	0.236	0.262	-
0.37	0.224	0.252	-
1.58	0.353	0.224	-
1.90	0.360	0.220	-

Table 2. Intrinsic viscosity vs. degree of substitution and salt concentration*. Charged sulfate groups along the chain will cause an expansion of the coil due to electrostatic repulsion. This will cause a change in intrinsic viscosity. This value will also be affected by the degree of dissociation into polyanions and counter-ions. At high salt concentration of e.g. 0.5M NaCl will cause neutralization of the electrostatic forces. The coil will then behave as if it were uncharged.*

Optical rotation

Whereas dextran shows very little change in optical rotation with ionic strength of the solvent, significant effects can be observed with charged polysaccharides (e.g. carrageenans). The measurements are not however dependent on concentration, but temperature may have a modest effect with certain sulfated polysaccharides. The specific optical rotations for a series of dextran sulfates with sulfur content in the range 17-19% are shown below in Table 3. It may be noted that the rotation values show a gradual increase with the exception of the 500000 derivative.

Mol. Wt. M_w	$[\alpha]_D$, degrees
5000	+ 72
10000	+89
20000	+93
100000	+99
500000	+86

Table 3. Optical rotation of dextran sulfate with sulfur content of 17-19%. *Measurements of optical rotation are not dependent on concentration. Temperature might have a modest affect.*

Storage and stability

The stability of dextran sulfate has been studied in both dry form and in solution. In dry form, a retrospective study showed that dextran is stable for more than five years when stored dry in well-sealed containers protected from light at room temperature. Opened containers should be sealed to prevent ingress of moisture.

To study the stability of DSS solutions in water, 2.5% solutions of DSS were prepared and tested as follows:

- 2.5% sterile, stored fridge 7 days.
- 2.5% stored room temp. 11 days.
- 2.5% stored room temp. 21 days

The results are summarized in Table 4 below.

Sample	Time (days)	pH	Free sulfate (mg/ml)	Mean mol. Wt.	Mw/Mn
1 (control)	7	6.54	0.03	42 000	1.4
2	11	6.25	0.03	41 400	1.4
3	21	5.50	0.03	41 900	1.4

Table 4. Summary of stability data of dextran sulfate sodium (DSS). *DSS was mixed with water to a final concentration of 2.5 %. All reported values are within the expected range. No degradation of DSS was seen during the 21-day study.*

All reported values lie within the expected range and did not provide any evidence of degradation of the DSS solution during the experimental interval studied (max. 21days).

A prolonged storage (3 months) study of solutions of DSS at room temperature revealed a drop in pH and a slight release of sulfate groups (< 1% of the total). Sterilisation is preferentially performed by sterile filtration.

Applications

Anti- coagulation agent in cell media and effect on cell proliferation

Dextran sulfate is a common additive in cell-media[4], were it is used as an as an anti-coagulant.

Cell clumping is a problem for several reasons:

- It obstructs accurate cell counting, cell monitoring and control of the cellular surroundings[5].
- Both transport of nutrients to the cell and transport of products originating from the cells may be impaired[5] .
- Cell clustering affects growth behavior dramatically[5]
- Aggregation reduces the rate of proliferation[5]
- Sheer forces cause a considerably higher death rate on aggregated cells compared to forces exerted on single cells [5]

Dextran sulfate 5 kDa has shown to diminish cell-aggregation during culture of Chinese hamster ovary (CHO) cells. A combination of 1.3 g/L DS and 8.0 mg/L r-trypsin was found to remove the cell aggregations completely [4].

In another study, DS was shown to inhibit apoptosis and increase protein production in CHO cells 1.9-fold compared to controls[6]. Dextran sulfate is a major ingredient in most types of commercially available cell media.

The value of polysaccharides and anionic derivatives in stem cell cultivation and extracellular matrix deposition has received much attention[7–11].

Precipitation of Lipoproteins

Dextran sulfate can be used to selectively precipitate lipoproteins. A mixture of 0.05 M dextran sulfate (M_W 10 kDa) and 0.05M $MnCl_2$ has shown to participate both VLDL (very low-density lipoprotein) and LDL (Low-density lipoprotein)[12]. A concentration of 0.65 % dextran sulfate and 0.2M $MnCl_2$ have shown to cause precipitation of HDL (High-density lipoprotein)[12]. Dextran sulfate (M_W 500 kDa) has been used to study HDL (High density lipoprotein) in similar ways[13].

3.3 Accelerate DNA hybridization and release of DNA from histones

Dextran sulfate can accelerate DNA hybridization. A concentration of 10 % Dextran sulfate has shown to increase DNA hybridization 10-fold in solution [14]. Studies have also shown that dextran sulfate can form complexes with histones and release DNA from DNA-histones complexes[15].

tRNA and ribonuclease effects

Dextran sulfate has been used to inhibit tRNA-binding to ribosomes[16]. It has also shown to inhibit ribonucleases[17].

Separation of microorganisms and macromolecules

In aqueous biphasic polymer separations, dextran sulfate has, in combination with polyethylene glycol, been used to separate viruses, bacteria, nucleic acids and proteins[18].

Anti-viral properties

Dextran sulfate has displayed anti-viral properties. One study showed that DS (10 kDa) can provide a complete protection of human T-lymphocytes against HIV *in vitro*. The effective concentration DS were 5 µg/ml. Dextran sulfate protects the cells by blocking the binding of viruses to the cell membrane[19]. Another study has however showed that the inhibitory potency of DS 10 against HIV varies considerably with cell type and virus strain [20]. In the same study, it is suggested that DS inhibits replication of enveloped viruses, e.g. etro-, herpes-, toga, arena-, rhabdo-, orthomyxo- and paramyxoviruses, but are ineffective against non-enveloped viruses such polio, Coxsackie and reovirus. Another study showed that DS 5, DS 8 and DS 500 can inactive the viral fusion protein in influenza A[21].

Dextran sulfate in Cosmetics

In the literature, it is suggested that Dextran sulfate possess the following cosmetic properties:

- Anti-aging
- Anti-wrinkle
- Anti-inflammatory
- Anti-allergic
- Moisture preservation
- Provides a smooth, fresh and "non-sticky" feeling
- Good for threatening of coarse and chapped skin
- Increasing of lipase activity by giving weigh-reducing effects and agile skin

The anti-inflammation priorities of dextran sulfate has been demonstrates in a number of studies[22,23]. There have been no detailed reports on the moisturizing effects of dextran sulfate. However, the osmotic retention of water of dextran sulfate present in tissue will contribute to the mechanical properties of the tissue concerned[24].

Studies of perm selectivity of membranes

The glomerular processing of charged and uncharged molecules in rats and mice has been studied by many research groups- and for this dextran sulfates of different molecular weights and degrees of substitution have been used. In general, one observes a lower clearance with negatively charged dextrans. It is presumed that the negatives charges of the endothelial glycocalyx and of wall-absorbed plasma proteins cause repulsion of the circulating large polyanions [11,25–28].

Studies of DS as Adjuvant in vaccines

Dextran sulfate sodium has become the gold standard for inducing colitis in mice. These strong inflammatory effects of DS has led to studies on DS's ability as a vaccine adjuvant[29].

One example is the study of the effect of high molecular weight dextran on cell-mediated immune response in mice and guinea pigs[30].
Footpad swelling, intra-dermal skin tests and macrophage migration inhibitory factor (MIF) production where used as criteria where used asses cell mediated delayed-type hypersensitivity. The study showed that the Guinea pigs who had been sensitized with egg-albumin and thereafter treated with DS showed strong positive delayed skin tests[30]. Mice lymphocytes, also sensitized with egg albumin and treated with DS, showed an increase in MIF production in the study. The DS also had an effect on footpad swelling in mice sensitized with sheep red blood cells[30]. The results from this study indicates that DS is a potent adjuvant for cell-mediated delayed-type hypersensitivity in Guinea pigs and mice.

In another study of high molecular weight DS effect on cell-mediated immune responses, it was showed that the adjuvant DS is capable of suppressing cell-mediated immune responses in mice and guinea pigs[31]. The criteria to asses cell-mediated immune response was skin graft rejection and intradermal skin tests. Mice that revived both allografts and DS showed a significant increase in graft survivals compared to controls[31]. Guinea pigs sensitized with Mycobacterium tuberculosis and treated with DS showed a reduced delayed skin test response[31].

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