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Specialists in Polysaccharides

Dextran sulfate

An anti-clumping agent in mAb producing CHO cell culture systems

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Introduction

Since decades, Chinese hamster ovary (CHO) cells have been employed as the predominant hosts to produce industrial recombinant proteins and therapeutic monoclonal antibodies (mAbs) [1,2]. This is because CHO cells can be adapted to grow in single cell suspension cultures at high densities (10^6-10^7 cells/mL) for large scale production of antibodies that can be readily scaled to over 10,000 L stirred tank bioreactors [3-5]. Further, CHO cell culture systems offer high productivity and human-like glycosylation profiles for monoclonal antibodies [6]. However, two main aggregation related challenges often arise during the cultivation process that hampers the cells themselves and the yield:

- **Cell aggregation:** In both the fed-batch and perfusion cultures, CHO cells frequently form aggregates [7,8] that reduces their overall growth, productivity, and induces apoptosis in dense cell clusters.
- **Antibody aggregation:** Secreted antibodies form aggregates or oligomers that compromise the product efficacy and stability, thereby increasing immunogenic risks in patients [9,10].

Aggregation of both the cells and antibodies can be effectively mitigated by addition of certain anti-clumping agents, in particular dextran sulfate. This report provides a comprehensive scientific review of dextran sulfate used in mAb production, covering its mechanisms of action, optimal usage strategies, effects on mAb yield and quality, potential drawbacks, considerations in industrial practice and regulatory contexts.

Cell aggregation

One of the most common and complex challenges faced in cell culture is the aggregation of cells that significantly compromises the reliability and reproducibility of experimental results. This is because when cells aggregate, it not only impacts the accuracy of cell counting but also greatly limits oxygen transport and nutrient diffusion between them leading to uneven growth and generating a heterogeneous cell population. So, some cells might proliferate vigorously while others face nutrient deprivation or enter dormancy, potentially resulting in cell death. Experiments requiring cells to interact with other cells or matrix could be negatively affected by the presence of aggregates, ultimately impacting the metabolic and secretory activities of the cells. So, cell aggregation impacts the overall behaviour of cells in terms of their viability, growth, proliferation, morphology, metabolism and functionality. Further, aggregation increases the complexity of handling and the risk of cell mortality during passaging that impairs the overall quality of the culture [11,12,13].

Cells can aggregate for various reasons. Exposure to external stress such as the non-optimal temperature of the culture medium or PBS, transporting cells at ambient temperature, and improper dissociation during passaging can cause the cells to detach from the culture flask and form aggregates. These aggregates can be exposed to enzymatic dissociation to form single cell suspensions, their viability checked by trypan blue staining and then re-seeded into

fresh culture vessels. Normal morphology could be restored once the cells begin to reattach and spread out [13].

Further, some suspension cell lines such as U2932 and AtT-20 naturally grow in small and large aggregates respectively. No special intervention is therefore required if aggregation is part of the normal growth pattern of such cell types [14,15]. In contrast, cell lines adapted to suspension cultures such as HEK293 and CHO-S are easily prone to aggregation at high cell densities resulting in premature cell death and significantly reducing the yields of protein expression. Cell aggregation in such cell types can be effectively mitigated by adding anti-clumping agents to the culture medium that aid in extending the cell viability under high-density conditions and enhance protein expression [13].

• Removing aggregation in CHO culture systems

Aggregation of both the CHO cells and monoclonal antibodies is undesirable. One approach is to physically resolve the aggregates by increasing the agitation rates in laboratory scale shake flasks just like that in the bioreactor. However, excessive speed not only fails to break up aggregates that are tightly bound but also leads to cell damage due to the concomitant high shear rates, thereby making the physical approach less than satisfactory [16].

A better alternative to mitigate the aggregation issues and maintain cells in single cell suspension cultures is by adding certain additives that serve as anti-clumping agents. For this purpose, polysulfated compounds such as suramin, sodium heparin, heparan sulfate, and dextran sulfate have been apparently used since the negative charge of these compounds alters the surface charge of cells leading to single cell suspension [17-22]. Amongst these, dextran sulfate, a polysulfated compound with a high density of sulfate charge has been widely used as a promising anti-aggregation agent in the suspension culture of CHO cells, where it not only stabilizes the culture conditions by primarily addressing cell aggregation but also indirectly affects the quality of antibodies produced [23]. In biopharmaceutical manufacturing, dextran sulfate has thus gained utility as a cell culture additive, especially in mAb production using CHO cells to improve culture performance and product quality. Once aggregation is resolved, nutrients and antibodies can diffuse freely further improving mass transfer in bioreactors.

Commercially, dextran sulfate is being supplied as an anti-clumping agent A by Lonza that is shown to alleviate aggregation and increase peak cell density in serum-free CHO suspension cultures [24]. Further, ExpiCHO Expression Medium supplied by Thermo Fisher's Gibco aids in high density growth of suspension-adapted CHO cells and enables high antibody yields, however the components within are highly confidential [25].

Case studies

For executing effective anti-clumping properties in CHO culture systems, the optimal molecular weight of dextran sulfate varies with one study showing 40 kDa to be highly effective while 5 kDa has also been widely used in other studies. Park et al., 2016 [26] demonstrated that the production and overall quality of mAbs depended on the molecular weight and concentration of dextran sulfate along with CHO cell lines. Dextran sulfate of 40 kDa was found to be highly effective in improving the growth and viability of CHO cells by attenuating cell aggregation along with increasing the mAb productivity (a chimeric antibody directed against the S surface antigen of the hepatitis B virus). Further, addition of dextran sulfate did not negatively impact the quality attributes of mAbs such as their aggregation, charge variation, and glycosylation.

Jing et al., 2016 [23] used a combination of 1.2 g/L dextran sulfate (5 kDa) and 8 mg/L recombinant trypsin to demonstrate effective neutralization of CHO cell aggregates that increased viable cell density and maintained the integrity of CMAB-802 antibody. Another study by Menvielle et al., 2013 [27] reported erythropoietin (EPO) expressing CHO cells being protected from apoptosis by dextran sulfate (5 kDa), that further extended their lifespan and significantly enhanced the productivity of EPO by 1.8 folds compared to controls. Further, Zhang et al., 2012 [28] reported the use of a 5 kDa dextran sulfate at 50 mg/L to effectively prevent clump formation.

Dextran sulfate: Critical parameters to consider

Dextran sulfate is an anionic polysaccharide produced by chemically sulfating dextran (a glucose polymer), resulting in molecules of varying molecular weights and sulfation degrees [29]. Dextran sulfate mimics endogenous glycosaminoglycans [27], and its strong negative charge enables virus inhibition [30], protein stabilization [31], and electrostatic interaction studies in biochemical systems [32], along with reducing aggregation in cell culture, thereby increasing viability and productivity [23].

While the above section mentions various studies undertaken with different concentrations and molecular weights of dextran sulfate, this section highlights the optimal range of both the concentration and molecular weight of dextran sulfate along with the degree of sulfation and timing of its addition that a researcher should be cautious about.

- ### Molecular weight and optimal concentration

The molecular weight of dextran sulfate ranges from ~5 kDa up to several thousand kDa [29]. Determining the right dose of dextran sulfate is extremely crucial, as its effect can be highly dependent on the concentration. In practice, dextran sulfate is effective at relatively low concentrations, while over-supplementation may prove toxic to the cell culture.

Low molecular weight dextran sulfates (5-15 kDa) diffuse easily and are often sufficient in preventing cell clumping at modest concentrations. Further, they also appear to be less inhibitory to cells. A lot of case studies mentioned above have used 5 kDa dextran sulfate, and so do most of the media formulation companies due to the proven balance of potency and less to no toxicity. The concentration of 5 kDa dextran sulfate indeed seemed to play a role as exceeding beyond 50 mg/L led to a decrease in cell viability and antibody concentration [28]. This could likely be due to the toxic or growth-inhibitory effects exerted by the excess polyanion where it sequesters essential growth factors or ions. In contrast, another study found no cytotoxic effects when 5 kDa dextran sulfate was used at a concentration of 50-250 mg/L [27]. This discrepancy in the optimal concentration could be possibly attributed to the specific characteristics of the cell lines used in these studies.

In the study led by Park et al., 2016 [26], it was shown that 40 kDa dextran sulfate was much more efficient compared to 4 kDa and 15 kDa in preventing aggregates and improving viability along with achieving highest peak mAb production in CS13-1.00 CHO cell line. In the other CHO cell line SM-0.025, 15 kDa dextran sulfate at 1 g/L was shown to enhance cell growth and viability but simultaneously reduced mAb production, indicating that higher concentrations do not necessarily lead to better outcomes. Similarly, antibody production in the same cell line decreased in the presence of 40 kDa dextran sulfate at concentrations of 0.3, 0.5, and 1 g/L, without any significant improvement in cell viability. High molecular weight dextran sulfate (40 kDa) thus showed contrasting results, where it was more effective in its anti-clumping properties for one cell line while it conferred higher toxicity in the other cell line. Optimizing both the molecular weight and concentration of dextran sulfate for each cell line is thus strongly recommended.

Moreover, irrespective of the molecular weights used (4 kDa, 15 kDa, 40 kDa), employing dextran sulfate at 2 g/L was found to be highly toxic as it decreased cell viability below 50% and irrespective of the concentration used (0.1-1.0 g/L), employing dextran sulfate with a molecular weight of 200 kDa was highly detrimental to CHO cells as it decreased their viability and titer of mAbs by 20-30% [26].

The toxicity of dextran sulfate is thus directly proportional to both its molecular weight [22] and concentration [33]. Ideally, researchers should systematically test gradually increasing concentrations of dextran sulfate starting from 0.1 g/L and check its effect on the growth and productivity of CHO cells. In practice, it may be sensible to start with a low molecular weight dextran sulfate at a low concentration and then gradually increase it. If clumping is still only partially resolved, then switching to a higher molecular weight dextran sulfate at a low concentration could be worthwhile as that could efficiently remove clumps. However, careful monitoring of cell health would be needed and optimal concentration might be re-titrated.

- **Degree of sulfation**

Degree of sulfation alters the charge density of the polymer, and it lies between 8-13% and 16-20% for low and high sulfated dextran sulfates respectively [29]. High sulfated dextrans carry a much higher negative charge, thereby maximizing their anti-aggregation capability as compared to the low sulfated dextrans. This is supported by the stronger anti-clumping effects observed for high sulfated dextran sulfates of 15 kDa and 40 kDa (17–19% and 15–19% sulfur content, respectively), compared with the weaker effect of low-sulfated (3–6%) 4 kDa dextran sulfate [26]. However, quantitative studies on the degree of sulfation of dextran sulfates in mAb production by CHO cells are sparse. Generally, most of the studies have used high sulfated dextran sulfate by adjusting their concentration rather than using a less-sulfated variant for efficiently preventing cell clumps.

- **Timing of addition**

The timing of adding dextran sulfate to cell culture is another important consideration. In mAb production processes, dextran sulfate is usually introduced at the start of the culture or early during the growth phase to prevent aggregate formation. For batch and fed-batch CHO cultures, dextran sulfate is commonly included in the basal medium at inoculation [28]. This ensures that as the cell density rises, dextran sulfate is present to keep cells from sticking together thereby sustaining anti-aggregation benefits. For instance, in one fed-batch process, CHO cells were seeded in dextran sulfate-containing medium and remained mostly single cells throughout growth, whereas the dextran sulfate-free control developed sizable aggregates early on [23]. If not added initially, dextran sulfate can be introduced during the culture when aggregation becomes noticeable (e.g. mid-exponential phase), but waiting too long could be generally less effective as it could be harder to disperse large clumps without harsher measures. However, it is important to remove or dilute dextran sulfate from cell media formulation used in transient transfection or viral infection (in HEK293 or insect cells) for virus or protein production. This is because dextran sulfate can bind to DNA/polycation complexes (like PEI–DNA) due to its negative charge, thus interfering with gene delivery [34].

Overall, for stable CHO cell line cultures producing mAbs, the simplest approach is to include dextran sulfate in the initial media preparation and maintain it throughout the production run. However, the concentration and molecular weight of dextran sulfate should be optimized for each cell line under study to effectively prevent aggregation of both the cells and product, at the same time not affecting the growth or metabolism of cells.

Mechanisms and advantages of dextran sulfate use in CHO cell culture

There are various advantages of incorporating dextran sulfate while culturing the suspension cells or suspension-adapted cell lines:

1. **Anti-viral properties:** Viral contamination can be kept in check due to the antiviral properties of dextran sulfate and this provides additional safety during cell culture [30].
2. **Non-adherent properties:** Dextran sulfate helps in detaching cells from the surface and maintain them in the suspension state [35,36]. Downregulation of cell surface adhesion proteins such as ICAM-1 and E-cadherin through activation of the Wnt signaling pathway, and upregulation of anti-adhesive factor such as TGFβ-induced by dextran sulfate could probably play a role [37]. A recent study also identified cell adhesion molecules such as integrins, cadherins, IgG superfamily and ECM proteins to be significantly enriched on the surface of aggregation-prone CHO cells, that could potentially be targeted in knockdown strategies to counteract aggregation [38].
3. **Anti-clumping properties:** Dextran sulfate is polyanionic and it not only repels cells from each other through electrostatic forces but also competitively blocks the cell adhesion receptors/substrates thereby keeping them in a single-cell suspension state and thus it prevents cell death related to shear stress in clumps. Addition of dextran sulfate thus ensures a homogenous cell distribution, especially in a serum-free environment thereby facilitating accurate cell counting and monitoring [22].
4. **Anti-apoptotic and Pro-autophagic effect:** Dextran sulfate inhibited apoptosis and promoted cell survival of EPO producing CHO cells through reduced expression of pro-apoptotic protein p53 and increased expression of pro-survival factor Hsc70 along with delaying DNA fragmentation and reducing the amount of Annexin-V positive cells. It also increased the formation of autophagosomes with an enhanced LC3-I to LC3-II conversion. This suggests that dextran sulfate may serve as an autophagy inducer facilitating the removal of misfolded proteins via autophagic pathways and improving the overall protein folding homeostasis. The net result of these effects is a longer-lasting, healthier cell population that can produce more antibody [27]. Further, dextran sulfate inhibited staurosporine-induced apoptosis in CHO cell cultures through suppressing the mitochondrial pathway-associated apoptosis [39].
5. **Cell viability and Cell density:** Dextran sulfate promotes overall cell growth and viability along with recombinant protein productivity [23,26].
6. **Quantity (Yield) of the product:** Dextran sulfate increases the productivity of recombinant proteins in CHO cells by reducing cell aggregation, improving cell viability and prolonging the culture lifespan. However, the overall quality and titre of monoclonal antibodies will be affected depending on the selection of CHO cell line, and the molecular weight and concentration of dextran sulfate. This was evident from one of the studies that not only the cell aggregation was effectively attenuated in CS13-1.00 cell line with the use of a 40 kDa dextran sulfate, but it also showed highest

maximal mAb concentration while SM-0.025 cell line exhibited a significant decrease in specific mAb productivity, particularly at a high concentration of dextran sulfate [26].

7. **Quality of the product:** It has been observed that dextran sulfate did not adversely affect the quality of mAb produced, rather it improved the quality attributes such as the aggregation state, purity, structural integrity, charge heterogeneity, and post-translational modification such as glycosylation. Further, the proportion of monomeric antibodies in the harvest increased due to the addition of dextran sulfate, indicating improved product homogeneity and quality [26]. Aggregation of antibodies was also reduced as dextran sulfate binds to partially unfolded proteins that can sometimes self-associate into aggregates during cell culture. Dextran sulfate can thus act in a *chaperone-like manner* by binding to such intermediates and preventing them from forming large aggregates. This mechanism could be explained from a study of dextran sulfate preventing heat induced aggregation of BSA protein. Notably, at a neutral pH, dextran sulfate does not bind to native BSA, but if the protein begins to partially unfold, the polyanionic chains of dextran sulfate can associate with the exposed cationic or hydrophobic regions on the protein. This "captures" the unfolding protein and stearically hinders multiple protein molecules from aggregating together due to electrostatic repulsion [31]. Aggregates of antibodies are undesirable because they can be immunogenic or reduce efficacy, so the ability of dextran sulfate to minimize aggregation during production is a significant advantage [9,10].
8. **Removal of Impurities:** Dextran sulfate can improve the purity of culture by reducing the release of host cell impurities. Since there is minimal cell aggregation due to the presence of dextran sulfate, cells undergo lower cell lysis due to less apoptosis and shear stress. This ultimately could lead to lower levels of DNA and HCP (Host cell proteins) in the harvest. Further, dextran sulfate likely binds to DNA and protein contaminants or alters their interaction with resin, thereby allowing their efficient removal according to a patent demonstrating that treating cell harvest with dextran sulfate significantly reduced DNA and HCP levels in the Protein A eluate [40].
9. **Enhanced protein transduction:** Dextran sulfate has also been shown to enhance protein transduction in CHO mutants that are deficient in the synthesis of either heparan sulfate or glycosaminoglycan mainly through electrostatic interactions, the important process by which the protein transduction domains and their cargoes traverse the plasma membrane [41].

Disadvantages and potential side-effects of dextran sulfate use in CHO cell culture

While dextran sulfate offers many benefits, it is important to acknowledge its limitations and potential side effects in both the cell culture and downstream process. Some known disadvantages include cytotoxicity at high levels that affect the overall growth, viability and productivity in some CHO cell lines and potential metabolic effects such as increased lactate accumulation due to dextran sulfate binding to an essential mineral and a heavy metal such as copper [26, 42, 43]. Apart from interaction with the genetic material during transient transfection or viral infection in HEK293 or insect cells [34], there could also be some challenges associated with the downstream processing of dextran sulfate. High levels of dextran sulfate might form complexes with DNA, which could increase viscosity or form precipitates. This is usually beneficial for impurity removal but if extreme, could affect mixing or aeration. Nevertheless, the side effects of dextran sulfate are manageable with appropriate dosing to prevent cytotoxicity and growth inhibition and ensuring its complete removal from the final product.

Safety and regulatory considerations

Though, dextran sulfate is essentially animal-origin free, dextran is primarily derived from fermentation of sucrose by *Leuconostoc* bacteria and further sulfated to generate the polysaccharide; dextran sulfate [29]. It can thus contain endotoxins or other impurities if it is not highly purified. So, to avoid introducing pyrogens, pharmaceutical-grade, endotoxin-tested dextran sulfate could instead be used in the commercial manufacture of mAbs using CHO systems.

The application of dextran sulfate in commercial mAb manufacturing must align with industry standards and regulatory requirements. Regulatory agencies like the European Medicines Agency (EMA) generally allows process additives like dextran sulfate, provided that its use is justified, controlled, and that it does not carry through to the final product at unacceptable levels. The EMA's guideline on cell-based medicinal products suggests that any biologically active additives (like growth factors, cytokines and antibodies) should be correctly documented with respect to their purity, identity, sterility and biological activity [44]. Dextran sulfate could be considered a biologically active additive due to its effect on cells, and thus its function and removal should be described.

Further, effective downstream purification is required if dextran sulfate is employed as it is considered a process-related impurity. This means residual dextran sulfate must be effectively removed to safe levels usually by additional washing or standard protein A and ion-exchange chromatography. Regulatory requirements will be satisfied when dextran sulfate is minimized or removed from the final yield and appropriate quality documentation is in place.

Conclusion

Dextran sulfate plays a pivotal role in enhancing the performance of upstream mAb production, particularly in suspension cultures using CHO and other mammalian cell lines. This report reviews the scientific and industrial rationale for incorporating dextran sulfate in cell media, focusing on its ability to prevent cell aggregation and stabilize secreted antibodies, thereby improving both the yield and product quality.

Through electrostatic repulsion and modulation of adhesion pathways, dextran sulfate maintains cells in single suspensions, improves mass transfer, and extends culture viability. It also exhibits chaperone-like effects where it minimizes antibody aggregation and reduces host cell proteins. When used at optimized concentrations and molecular weight, dextran sulfate has been shown to significantly increase viable cell density and antibody titers without compromising the product quality.

While dextran sulfate must be carefully dosed to avoid cytotoxicity or downstream complications, its advantages have led to its widespread adoption as an additive in CHO cell media/culture for both research and industrial mAb manufacturing. Its use improves process robustness and productivity without compromising regulatory compliance or patient safety.

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