Characterizing the Size and Molecular Weight Distribution of Starch: Why It Is Important and Why It Is Hard

- Starch is the largest component of our food energy and has important industrial applications.
- Better foods and improved industrial applications require structure-property relations for starch.
- However, starch is a huge and highly branched molecule, and there is no general agreement on the best ways of characterizing this structure. This article discusses the problems, current best practices, and the way forward.

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Starch is one of the most important polymers for humanity: It comprises the largest single component of our food energy, and is also an important industrial polymer, for example, it is used in papermaking, biofuels, and the food and pharmaceutical industries as an additive with diverse uses. On one level, it is simple: a polymer of glucose (or of anhydroglucose, to be more precise). However, it is a mix of two polymer architectures: amylose—largely linear with a few long-chain branches—and amylopectin, which is highly branched. Because of this branching, it shows a bewildering structural complexity. Starch structure (including amylose to amylopectin ratio and other structural features) dictates starch properties, such as how rapidly and where a food is digested, or how well a modified starch might serve as a thickener or dispersing agent. The cereal food industry commonly uses molecular weight as a means of quality assurance. However, things are not that simple.

First, "molecular weight" in this context usually means an average such as the weight-average molecular weight ($\overline{M}_w$). However, any starch sample contains a vast range of molecular weights and the distributions of these molecular weights can be important. Two starches with the same $\overline{M}_w$, but with very different distributions, could have quite different rheological properties. Average size is not everything.

Second, devices commonly used for measuring molecular weight, especially size exclusion chromatography (SEC, also known as gel permeation chromatography [GPC]), use separation by size, not by molecular weight. Starch is a branched molecule and two molecules with the same size can have different molecular weights if their branching structure is different. Thus, for starch, there is no unique correspondence between size and molecular weight. Some popular instrumentation packages purport to give molecular weight distributions, whereas in actuality, they produce a more complex quantity.

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Third, measuring molecular weight or molecular size distributions requires that the starch be fully dissolved (dispersed as separate molecules) without degradation. As will be seen, that is often a problem.

Fourth, there are huge discrepancies on starch molecular weights in the literature, especially for amylopectin. A literature search for "molecular weight of waxy maize starch" gives results ranging from 10^6 to 10^8. Clearly, this situation is completely unsatisfactory.

Thus, not only is molecular weight characterization a problem, but many practitioners are in fact not characterizing what they think they are!

To this end, the International Union of Pure and Applied Chemistry (IUPAC) has set up an international task group on "Critically evaluated techniques for size separation characterization of starch," whose aim is to reach an agreement among world experts on optimal characterization technologies. This group includes specialists in food science and size-separation analytical chemistry and is drawn from industry (including starch companies and manufacturers of analytical equipment) and academia. As a first step toward its technical goal, the group has established what the various problems are (6) as a prelude to working toward their solution. Solving outstanding problems is important for producers, manufacturers, and consumers.

What Does Industry Need to Know and Why?

The importance of both the average molecular weight and radius of gyration of starch has long been appreciated by industry. Here are some examples.

For beverage thickeners, molecular weight that is too high results in a drink that is too viscous with a slimy or chalky texture, while a molecular weight that is too low results in a beverage with insufficient body (16). For resistant starch, the molecular weight needs to be in the correct range for suitable digestive properties and control retrogradation (15). To exemplify an important mining application, in the recovery of alumina from bauxite, the starch needs to be at least 2×10^6 in molecular weight, with too low a value resulting in a poorer quality of aluminum from the processing (13). In healthcare, starch is used in enteral nutrition solutions; too low a molecular weight results in browning problems and a too rapid release of calories, while too high of a value could result in the clogging of the tubing and thus a reduced calorie supply (2).

Although these are just a few of many examples in which these properties are important to a product or process, the key question remains: What is the molecular weight of the starch? Depending on how this is measured, the result may be different, so how do we obtain a reliable, and more importantly, accurate, value? Moreover, there is virtually no knowledge or understanding of how the distributions, rather than just average values, affect properties of interest.

Doing the Right Measurements with Current Knowledge

Many techniques used by industry can yield results that are meaningful for the desired goal, given current best-practice implementation.

- To dissolve all of the starch without degradation, starch must be dissolved completely and molecularly to characterize its size and molecular weight. While dissolving a starch sample is easy (e.g., boiling in concentrated NaOH for a long time), some facile procedures result in degradation. Fortunately, some starches are easy to dissolve without degradation, especially modified starches used for many industrial purposes (indeed, ready dissolution is often one of the reasons for the modifications).
- Meaningful molecular weights can be measured with care under the right circumstances. $\overline{M}_w$ can be measured by light scattering provided that steps are taken to ensure complete dissolution of the sample without degradation (6). Obtaining and processing light-scattering data suitable to yield $\overline{M}_w$ requires particular care (1). For example, it is necessary to ensure that the concentration is in an appropriate range (dilute, but not too dilute) and that the data treatment uses the correct value of the refractive index increment (how refractive index varies with polymer concentration [dn/dc]) in the particular solvent and temperature. $\overline{M}_w$ can also be obtained using SEC, but here the problem of degrading the amylopectin component through shear scission in SEC is extremely hard to avoid (4). Thus, SEC can be used to find $\overline{M}_w$ only for smaller starch molecules (amylose and degraded starches).
- Do distributions matter? It is reiterated that industry currently uses average molecular weights, not their distributions. It would not be surprising if distributions as well as averages were important for quality assurance, which needs case-by-case testing.

It is also essential to recall that SEC does exactly that: it separates by size, not by molecular weight. Because there is no unique relation between these two quantities for a branched polymer such as starch, in general, a given elution slice will contain a range of molecular weights. Light-scattering detection gives an absolute molecular weight, but this quantity is an average $\overline{M}_w$. SEC data, which include light-scattering detectors, can be manipulated with manufacturer-supplied software to plot distributions as a function of molecular weight, but a) this is the average $\overline{M}_w$; and b) it is only the true molecular weight if the correct dn/dc is available.

Despite these concerns, the use of data for a series of samples for comparative purposes among samples of similar type is still very useful, even though the absolute values may not be correct.

Solving Current Problems

Solubilizing Starch Without Hurting It

Characterizing the molecular weight and/or size (distributions) of starch requires that the characterization process fully dissolves the starch as separate molecules and does this without degrading them. While processed or modified starches can be easy to dissolve without damage (unless the modification involved covalent cross-linking), it is a different story with native starch. The granule itself consists of a layered semicrystalline structure. In order to extract the starch for analysis, the granule first needs to be broken down, then the starch itself needs to be dissolved.

Many techniques exist to extract and solubilize the molecules from starch granules. Aqueous or organic (dimethylsulfoxide) solvents can be used for this purpose, with or without salt ions (LiCl, LiBr). Alkali dissolution methods have been used previously, as has extraction using urea. The method of dissolution—thermal (high temperature, microwave) or mechanical treatment (sonication, stirring, shaking)—also needs to be considered. Amylopectin is a huge molecule and prone to shear scission. It has not yet been established which, if any, of the techniques currently in use can fully dissolve native starch as isolated molecules without degradation. The IUPAC task group has set this as its first problem to solve.

Imperfect Separation

Size separation is never perfect in practice: the eluent in a given narrow "slice" will contain a (hopefully narrow) range of different sizes. An example of this is band broadening in SEC, where this imperfect behavior arises from molecular diffusion, multiple flow paths along the column, etc. (3,12). With modern separation techniques and equipment, this effect is relatively

small, and has only minor effects on average molecular weights and the trends in distributions. Band broadening needs to be taken into account only in specialized comparisons of distributions with postulated functional forms.

In addition, some authors use the term "incomplete separation" to refer to the fact that size separation does not separate by molecular weight and, therefore, for a branched polymer, there will be a range of molecular weights in a given size slice, even with perfect separation. "Incomplete" is not a useful term here, because it is an effect inherent in the physical technique and, for branched polymers, is always present even if the separation technique is perfect.

Size or Molecular Weight?

The size of a branched polymer in solution can be defined in a number of ways, the most common of which is the radius of gyration (R_g). This and any other size measurement is an average of all of the different branching structures and conformations adopted by the molecules in the sample. Different techniques separate by different size parameters (and the separation parameter can also vary with the mode of operation of a given technique). Confusingly, these separation sizes are always defined as hydrodynamic volume (V_h), whose IUPAC definition (8) explicitly depends on the separation technique. For SEC, V_h is proportional to the product of intrinsic viscosity and number-average molecular weight (M_n) (11). However, for some types of field-flow fractionation, it is the center-of-mass diffusion coefficient (5), related to a corresponding radius by the Stokes-Einstein equation. While all of these "sizes" are proportional to R_g, with a proportionality factor of the order of unity, the actual value of this factor depends on the polymer architecture (and possibly on the size as well), and is in general unknown for branched molecules such as those found in starch. Because light scattering gives R_g for a polymer unambiguously, these relations can be determined directly (14), but instrumental limitations often preclude obtaining an accurate value, e.g., for smaller chains of amylose. Furthermore, the size of a starch chain in solution depends on the eluent and the temperature, as well as the sample.

While the $\overline{M}_w$ of starch can be measured unambiguously by light-scattering techniques, these techniques cannot give distributions from a sample (unless that sample has first undergone size separation)—there is no unique solution for the inversion of light-scattering data from a disperse sample to a size distribution. Such inversions can only be undertaken making various assumptions as to the nature of the distribution. Some devices (such as those which have a means of operating in so-called research mode) enable different models and assumptions to be tested and if the distribution generated is not too dependent on the type of assumptions made, it is semiquantitatively reliable. Unfortunately, some devices on the market include software that yields a purported distribution, but do not permit the underlying assumptions to be tested, and in such cases, while the averages from these devices are reliable, conversely, the distributions are not.

Size-Separation Methods

Size Exclusion Chromatography

Size exclusion chromatography (SEC or GPC) is a deservedly popular method for determining averages and distributions of both size and molecular weight. For the amylose component of starch, this works very well (as long as the columns and operating conditions are carefully selected), but for the amylopectin component, shear is very likely to occur (4). This gives a false distribution for the amylopectin region.

Asymmetric-Flow Field Flow Fractionation

Asymmetric-flow field flow fractionation (AF^4) is available commercially and relies on the diffusion of molecules and particles against a flow field into parabolic flow inside a channel. The lack of a stationary phase in the channel removes the effect of shear scission present in SEC; however, due to the porous nature of the membrane, some material could be lost through these pores. In addition, there can also be an interaction between the molecules and the membrane material, resulting in nonideal separation.

Thermal Field Flow Fractionation

Thermal field flow fractionation (ThFFF) is similar to AF^4 in that the separation is achieved by the diffusion of molecules against a flow field, except that for ThFFF, the field is generated by the temperature difference between a hot and a cold surface. This method removes the requirement for a membrane, and so removes the lower size cut-off. However, the technique needs optimization for starch (e.g., to avoid aggregation on the cold wall), and the separation parameter (size parameter) for ThFFF has not been established; determining this is another task for the IUPAC task group.

Hydrodynamic Chromatography

Hydrodynamic chromatography (HDC) is a technique (for which instruments, but not starch-optimal columns, are available commercially) that can be considered somewhere between SEC and AF^4. A stationary phase is present inside the column, as with SEC, with the separation of the molecules occurring by the diffusion of molecules into different flow streams. Seeing how this technique can be useful for the size separation of starch is an active area of research by various groups.

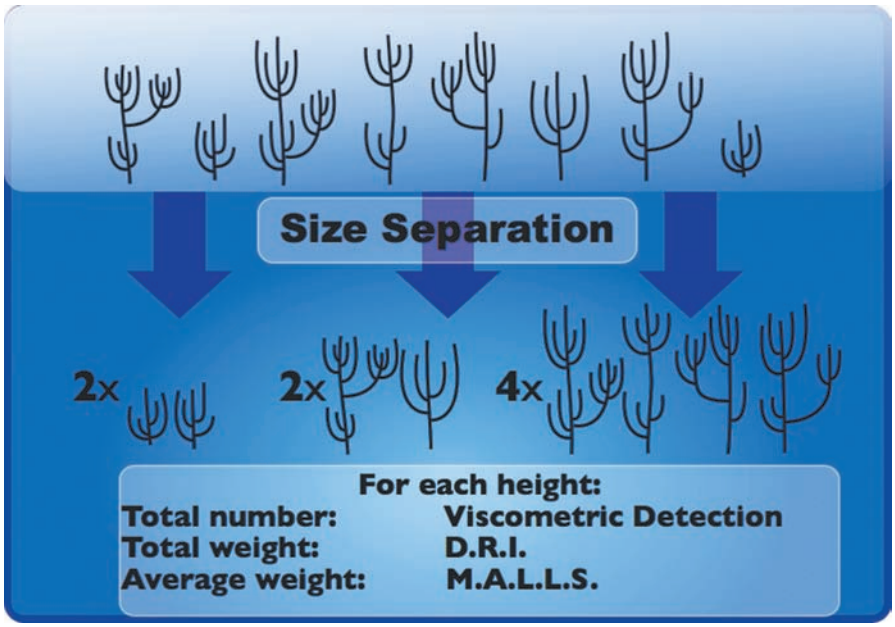

Fig. 1. Characterizing starch, a highly branched molecule, is analogous to characterizing the trees in a forest by first separating by size (or height, for trees), then using three separate detectors to find, for the trees of each height, the number (distribution), the total weight (distribution), and the weight-average weight. (In the figure, DRI is differential refractive index and MALLS is multiple-angle laser light scattering.)

Analytical Ultracentrifugation

Analytical ultracentrifugation (AUC), also available commercially, has been used for some time for size separation of starch, but the method has limitations in both establishing the size separation parameter in molecular terms and in poor separation (different sizes leaking into the same zone).

The comparison between and the applicability of these different techniques is again a priority for the IUPAC task group. In addition, a major task is to identify causes of variability, such as the presence of nonstarch components, poor size separation, aggregation, incomplete dissolution, incomplete separation (e.g., the exclusion limit of an SEC column), and shear scission.

What Do the Detectors Tell Us?

Aside from physically separating or fractionating the samples by a property related to their size using any one of the above techniques, it is also important to consider the detection methods available. Three types of detectors are widely used: differential refractive index (DRI), multiple-angle laser light scattering (MALLS), and in-line viscometry. For linear polymers, these effectively give the same information (apart from instrumental limitations); however, for a branched polymer such as starch, the information from the three detection types is complementary—the more types of detector, the more information about the system.

For branched polymers, even if one has perfect size separation (whatever the separation parameter might be for a particular technique), there will still be a range of molecular weights and branching structures of chains in a size slice. It is analogous to characterizing the trees in a forest. Supposing one separates the trees by height ("size" for the purpose of illustration). A collection of trees with the same height will have a range of branching structures and thus weights (Fig. 1). Different detectors then can give further information about the trees in this range. The number distribution of chains (equivalent to the total number of trees of a given height) is given by the viscometric signal (7), while the weight distribution (the total weight of trees with a given height) is found from the DRI detector. The average weight of trees of a given height ($\overline{M}_w$) is found from MALLS.

For commercial and scientific uses of starch, the application of these different types of distributions for biosynthesis-structure-property relations is just starting to be explored, and has considerable future potential for value-added commercial products and processes.

Further separation by other separation parameters is starting to become possible (so-called two-dimensional techniques [9,10]), but as yet unachieved for starch and unlikely to be ready for commercial use for some time.

What Do We Do Now?

Many questions exist as to what needs to be analyzed for a particular purpose, and what information in this regard can be obtained using the various techniques. There is no best technique for starch, because the optimal technique depends on what is needed for a particular purpose. Some significant technical issues are outstanding, many of which have been listed above as goals for the IUPAC task group working on this project.

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