Journal of Polymer and Biopolymer Physics Chemistry, 2018, Vol. 6, No. 1, 39-44
Available online at http://pubs.sciepub.com/jpbpc/6/1/5
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DOI:10.12691/jpbpc-6-1-5

Molecular Weight Distribution for Biopolymers: A Review

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Received October 10, 2018; Revised November 14, 2018; Accepted November 21, 2018

Abstract In this study, molecular weight distribution (MWD), of polymers with emphasis on MWD of biopolymers, e.g, carbohydrate polymers, proteins, deoxyribonucleic acid, DNA, and ribonucleic acid, RNA, are reviewed. The MWD of biopolymers are compared with those of synthetic polymers. The constitution of a polymer as well as the MWD may be described either by a set of different average molecular weights, the ratios of two different types of average molecular weights, or by the distribution functions via graphical presentation. Polysaccharides in a similar way to synthetic polymers are polydisperse polymers, whereas proteins, DNA, and RNA, are mostly monodisperse macromolecules.

Keywords: *biopolymers, molecular weight distribution, proteins, polysaccharides, DNA, RNA*

Cite This Article: Mohammad R. Kasaai, "Molecular Weight Distribution for Biopolymers: A Review." *Journal of Polymer and Biopolymer Physics Chemistry*, vol. 6, no. 1 (2018): 39-44. doi: 10.12691/jpbpc-6-1-5.

1. Introduction

Natural polymers, are produced by biosynthesis in nature, whereas synthetic polymers are man-made polymers and their synthesis controlled by human beings. Biopolymers may be classified into proteins, nucleic acids, polysaccharides, and others. In this manuscript, three main groups; polysaccharides, proteins and nucleic acids, which play important roles in all biological phenomena and processes are discussed [1,2,3]. A wide variety of natural polymers relevant to the field of biomaterials, is derived from plants and animals [2,4]. Generally, biopolymers consist of carbohydrate polymers, proteins, deoxyribonucleic acids (DNA), and ribonucleic acids (RNA). They are fundamental to the biological substance of life [2]. Abbreviations and expressions are listed in Table 1.

Table 1. Abbreviations used in this manuscript

Abbreviation	Expression, Term
CLND	Chemi-Luminescent Nnitrogen Detector
Da	Dalton
DI	Dispersity Index
DNA	Deoxyribonucleic Acid
DRI	Differential Refractive Index
GPC	Gel Permeation Chromatography
IUPAC	International Union of Pure and Applied Chemistry
RNA	Ribonucleic Acid
SEC	Size Exclusion Chromatography
MS	Mass Spectrometry
MWD	Molecular Weight Distribution

The properties, functions and applications of polymers depends on their molecular weight distribution (MWD). A Polymer with naroow MWD is used as standard polymer samples in size-exclusion chromatography (SEC) for determination of MWD of other polymers. An overview of molecular weight distribution (MWD), of polymers with emphasis on MWD of biopolymers, e.g, carbohydrate polymers, proteins, deoxyribonucleic acid, DNA, and ribonucleic acid, RNA, are presented in this study.

2. General Considerations

Proteins are linear polymers formed by linking the α-carboxyl group of one amino acid to the α- amino group of another amino acid with a peptide bond. A polypeptide chain consists of a regularly repeating part of amino acids joined by peptide bonds. Most of natural polypeptide chains contain between 50 and 2000 amino acid residues and are commonly referred to as proteins. The M_w of most proteins lies between 5.5 kDa and 220 kDa [4]. Constant and symbolsused in this study are presented in Table 2. Polysaccharides can be defined as linear or branched macromolecules formed by many monosaccharide units linked by glycosidic bonds. These biopolymers, sometimes also called glycans, can be classified as homo-polysaccharides, i.e. homopolymers, which consist of monosaccharide units, and hetero-polysaccharides, i.e. copolymers which consist of two or more different monosaccharide units [5]. The glycosidic bonds can be α or β (1→4, 1→6, 1→3, for instance). Depending on their functions, they can be also classified as structural, such as cellulose and chitin, and storage, like starch, inulin and glycogen [6].

Proteins, DNA, and RNA are linear polymers. DNA and RNA are nucleotide polymers and called nucleic acids [7]. However, proteins are more complex than DNA and RNA. Proteins are formed from a selection of 20 building blocks, called amino acids, whereas DNA and RNA are formed from four monomer units; nucleotide units [4]. Proteins, DNA and RNA with different types of monomers are also classified as copolymers [2]. The structure of a protein depends on the sequence in which individual amino acids are connected. All proteins are polypeptides. A protein has a polyamide backbone with different side chains attached to the backbone. A nucleic acid has an alternating sugar-phosphate backbone with a different amine base attached to it. The structure of a nucleic acid depends on the sequence of individual nucleotides [8].

Table 2. Constants and symbols used in this manuscript

Symbol	Definition
M	Molecular weight
M_w	Weight-average molecular weight
M_n	number-average molecular weight
M_z	z-average (centrifuge average) molecular weight
M_{z+1}	(z+1)- average molecular weight
M_v	viscosity-average molecular weight
[η]	Intrinsic viscosity in a solvent
K_{av}	Distribution coefficient
V_R	solute elution volumen
V_0	void volumen
V_C	total bed volumen

DNA and RNA consist of a large number of linked nucleotides, each composed of a sugar, a phosphate, and a base. Sugars linked by phosphates form a common backbone, whereas the bases vary among four different kinds [4]. RNA like DNA is long linear (long un-branched) polymers consisting of nucleoides joined by 3 to 5 phosphodiester. In both DNA and RNA, the heterocyclic amino base is bonded to C1' of the sugar, whereas the phosphoric acid is bonded by a phosphate ester linkage to the C5' sugar position [8].

The structure of RNA differs from that of DNA in two respects. The sugar units in RNA are riboses, and the sugar in DNA is 2'- deoxyriboses [4,8]. The other difference is that one of the four major bases in RNA is uracil (U) instead of thymine (T). Thus, four monomer units in DNA are adenine, cytosine, guanine, thymine, whereas in RNA are adenine, cytosine, guanine, and uracil [4]. The sugars in nucleic acids are linked to one another by phosphor-diester bridges (with negative charges). The chain of sugars linked by phosphodiester bridges is referred to the backbone of the nucleic acids. Whereas the backbone is constant in DNA and RNA, the bases vary from one monomer to the next. Though chemically DNA and RNA are similar, DNA and RNA differ in size. Molecules of DNA are enormous. They have molecular weights of up to 150 billion and length of up to 12 cm when stretched out, and they are found mostly in the nucleus of cells [8]. In contrast, molecules of RNA are much smaller as low as 35 kDa in molecular weight and are found mostly outside the cell nucleus [8].

3. Concept of Dispersity and Molecular Weight Distribution

The constitution of a polymer system is described either by a set of different average molecular weights or by the distribution [9]. Nearly all synthetic macromolecules are polydisperse polymers, due to the random nature of the polymerization reactions by which they are formed [1]. In nature, some macromolecules occur naurally as polydisperse samples. Therefore, large molecules of a polymer sample may also contain molecules relatively smaller and larger than the intermediate size. Hence, all polymers are more or less heterogeneous with respect to molecular weight.

The degree of dispersity i.e. dispersity index (DI) or dispersity is a new term for the previous terms “polydispersity” and for the original term “poly-molecularity” [5,10]. The new term was given by the International Union of Pure and Applied Chemistry (IUPAC). In the following, in order to avoid confusion, the term “dispersity” replaces polydispersity. The DI is generally expressed as the ratios of two different types of average molecular weights. Various average molecular weights will have the same value if the polymer is perfectly monodisperse, *i.e.*, if all the molecules are of the same molecular weight. Otherwise, the averages vary in the following order as given in the following equation as well as it is illustrated in Figure 1 [11-13]:

$$M_n < M_v < M_w < M_z < M_{z+1} \quad (1)$$

The disparity between average molecular weights provides a measure of the degree of heterogeneity in the molecular weight distribution [14]. The z, and (z+1) averages are important for very broad polydisperse polymers [15].

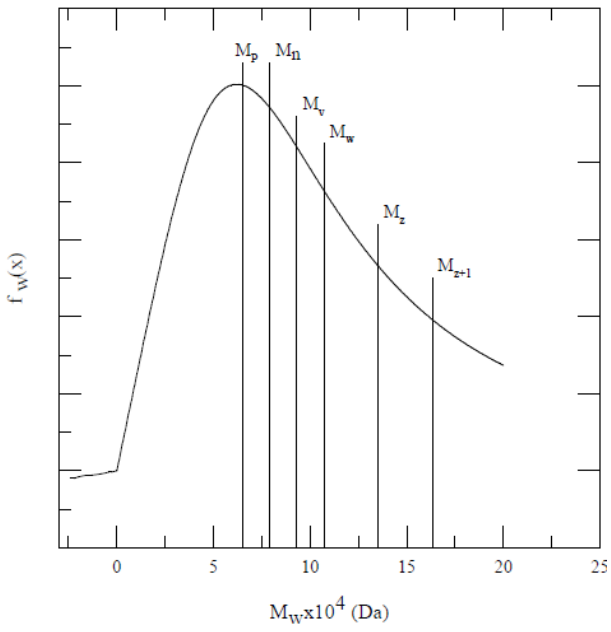

Figure 1. A typical molecular weight distribution profile with a semi-qualitative comparison 126 of different average molecular weights

The molecular weight distribution (MWD), is generally expressed as the ratios of two different types of average molecular weights. Thus, it is necessary to determine

different average molecular weights. It is useful to calculate two dispersity indices (DI_1, DI_2) as follows:

$$DI_1 = I_1 = M_w / M_n \quad (2)$$

$$I_2 = I_2 = M_z / M_w \quad (3)$$

The two indices can be used to evaluate the level of heterogeneity of different samples of a polymer [16]. The DI_2 is sensitive to the presence of the high molecular species. The DI is usually obtained from the ratio of M_w/M_n [5,11,17]. For mono-dispersed polymers the indices are close to unity, and the higher the indices, the greater the difference between the average weights and the larger the distribution [16]. In the case of monodisperse polymers, the average molecular weights are determined by different methods, likely to coincide and reach an identical value [18]. For instance, in the mono-disperse system, $M_w = M_n$. The molecular weights of samples with a wide MWD may differ by a factor of more than two.

Chemically heterogeneous macromolecules are polymers that contain units of different composition in the same chain. For example, some of the units may be completely esterified, while others may contain free hydroxyl groups. The chemical composition of such polymers is conventionally characterized by the average percentage content of their functional groups, e.g. acetyl [18]. Copolymers are more complex than most of homo-polymers. The chemical composition of a copolymer may not be uniform. Non-uniformity results in compositional heterogeneity [18], which is required to characterize the copolymers in terms of chemical compositional distribution, usually fractionation followed by chemical characterization of the fractions [17]. Chemical composition and size, i.e. molecular weight of a copolymer chain may vary [17]. Two polymers may have exactly the same or similar average molecular weights but very different MWDs [17].

3.1. Determination of Molecular Weight Distribution

There are several ways to measure MWD: (1) gel permeation chromatography (GPC) or size exclusion chromatography (SEC) has been used for the determination of molecular weight and molecular-weight distribution of polymers [12,18,19]; (2) fractionation of a polymer with a broad MWD into narrower MWD fractions and determination of molecular weight of the narrow fractions [20]. Fractionation is helpful in evaluating the true range of dispersity of polymers with a narrow MWD [21]; and (3) the molecular weight distribution curve of a polymer can be also obtained directly from the data on sedimentation of a disperse polymer sample using an ultracentrifugation procedure [18].

3.2. Size Exclusion Chromatography

SEC has been used for many decades to estimate M and MWD, and DI of biopolymers and synthetic polymers through the use of calibration curves between molecular weight and the distribution coefficient, K_{av}.

$$K_{av} = \frac{V_R - V_0}{V_C - V_0} \quad (4)$$

where V_R, V_0, and V_C represent solute elution volume, void volume, and the total bed volume of fluid and SEC media combined, respectively [22]. For the determination of the retention volume, it is much better to use the area method, the center of mass, than that of the height method, the peak maximum [23]. The values of A_i or h_i and V_{Ri} are read directly from the chromatogram. The values of V_{Ri} can be converted into M_i.

The chromatographic method separates the molecules according to their sizes. The larger is the molecule the greater is the exclusion from various sized pores in the stationary phase material. Accordingly, the higher is the molecular weight, the lower is the elution volume. Figure 2 shows three SEC chromatograms, A, B, and C, representing the highest, intermediate and the smallest macromolecules, respectively. Monodisperse polymer standards are required to translate elution volumes to molecular weights. From the SEC distribution curve, the various molecular weight averages may be also calculated [11,24,25].

The separation is based on the hydrodynamic volume of a polymer molecule. The hydrodynamic volume is proportional to the product, [η].M. The molecular weights of polymers do not correlate linearly with retention volumes, because retention volume is a function of effective hydrodynamic volumen, hydrodynamic volume [η].M, correlates with retention volumen, i.e. elution volume, Ve [17,26]. In order to construct a universal calibration curve of the hydrodynamic volume of a polymer as a function of the elution volume; *Ve*: the intrinsic viscosity and SEC chromatograms of well-characterized mono-disperse polymer samples are measured [19]. Coupling of a SEC with an automatic capillary viscometer results in more accurate data for the dispersity indices than SEC alone [24,27]. The coupling method also enables one to determine the resolution factor for a given SEC separation, column, system employed [27].

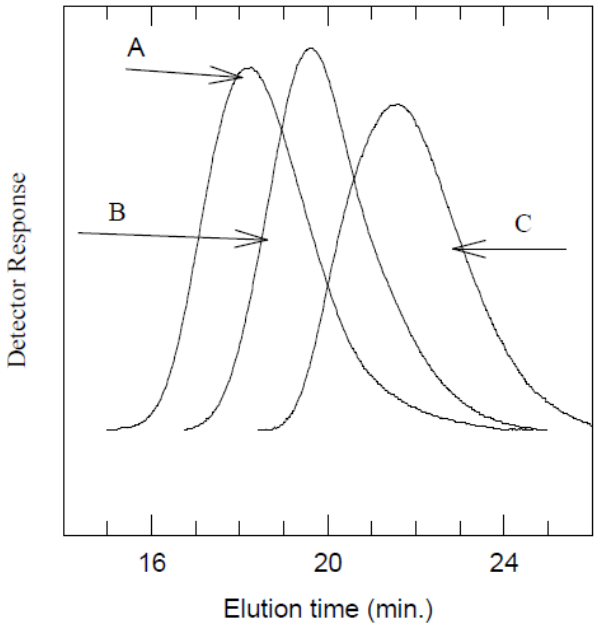

Figure 2. Three SEC chromatograms, A, B, and C, representing the highest, intermediate and the smallest macromolecules, respectively

Analyzing a complex mixture of unknown macromolecules is often challenging in GPC/SEC. GPC/SEC is used to

separate, identify and characterize macromolecules with respect to their MWD. The precision and accuracy of the results depend on the selection of the proper separation columns. GPC/SEC separates macromolecules based on their hydrodynamic volume and, therefore, allows macromolecule chains with different lengths to be separated into small fractions. However, MWD determined by SEC can be influenced by a number of factors. Peak-broadening effects and incomplete resolution can give misleading information [17].

Qualitative determination of MWD is a difficult task. SEC instrument with suitable standard samples would result in a qualitative evaluation of MWD for polymer samples. However, quantitative evaluation of MWD is more difficult than that of a qualitative one. Combining SEC with light scattering or another absolute method for molecular weight method is an alternative solution for the quantitative evaluation of MWD [13,28].

Generally, silica- and polymer-based materials are used as packing materials in SEC columns for determination of molecular weights and MWD of polymers. If silica-based materials were used to separate biopolymers such as pullulan, there should be interaction between silanol groups of silica-based packing materials and biopolymers. The interaction could perturb the validity of the calibration curve for the SEC process, and thus results in deviation for molecular weight data [29]. Generally, silica-based packing materials are chemically modified to remove the effect of the silanol groups, which tend to have a negative effect on biopolymer separations [30]. The polymer-based packaging materials have advantages over silica-based, due to lack or negligible negative effect on biopolymer separations.

Differential refractive index (DRI) is the most common means of mass detection for the MWD analysis of polymers by SEC. A disadvantage of DRI, is that this detector only provides concentration information and no information about composition and heterogeneity. A chemiluminescent nitrogen detector (CLND) with SEC was developed to estimate average MWD of peptides and food grade protein hydrolysates, as well as protein hydrolysate-based food. The DRI/CLND SEC analysis can detect differences between two lots of a polymer which have similar MWD, but dissimilar chemical composition distributions. Mass spectrometry (MS), provides structural information, differentiating molecules with small differences in molecular weight.

Pullulan is used as a standard in SEC, to determine the average molecular weights (M_n, M_w and M_z) and MWD for linear biopolymers by constructing a universal calibration curve. Up to date, there is no commercially available β-glucans or other similar polysaccharide standards that are comparable with pullulan with respects to the narrow dispersity. Generally, in order to have a good resolution as a function of molecular size, an appropriate solvent should be selected for the investigated polymers, polysaccharides or water-soluble polymers [21].

Fractionated dextrans have been also used as standard materials for M_w and MWD determinations of biopolymers and water-soluble polymers as well as for construction of universal calibration curve for evaluation of SEC results.

3.3. Fractionation

Generally, the composition of a polymer substance is not homogeneous. MWD is a general feature for all synthetic polymers and polydisperse biopolymers. It is a consequence of the particular nature of the polymerization process by which synthetic polymers are made [36].

Biopolymers are usually formed in nature via biological/biochemical processes. In nature, some of macromolecules occurr commonly as polydisperse materials. Biopolymers are also susceptible to degradation under environmental conditions like temperature, humidity, oxygen, light, and others. Biopolymers may react with other biomaterials through biochemical and physical reactions. Thus, the size of biopolymer species formed in nature yields also polydisperse materials. However, some biopolymers may occurr as relatively monodisperse samples.

Fractionation of a polymeric substance means separation of that substance into its different molecular species, using a suitable experimental technique, in order to obtain homogeneous fractions [36]. The disparity between different average molecular weights may be made small by careful fractionation [14]. Fractionation techniques separate polymers based on molecular weight or chemical composition. Practically, partial separations by molecular weight and chemical composition are often achieved simultaneously [17]. It is possible to separate and characterize a complex sample containing homo- and copolymer species based on chemical heterogeneity and molecular weight [17]. By fractionating a polymer and determining the molecular weights of each fraction, the MWD curve can be obtained. Differentiation of the integral curve gives the differential distribution curve. The basic characteristics of a differential curve are the position of its peak and its width. The broader the distribution curve the wider the molecular weight distribution [18].

Fractioination is an experimental procedure to separate a poplymer sample containg different species based on their sizes or compositions [37]. The fractionation methods include fractional precipitation, fractional distribution between two phases, fractional dissolution, and fractional extraction [37]. In the fractional extraction, fractional solution or fractional elution, the polymer sample is successively extracted with a solvent, whose power gradually increases. The residues are removed at each stage and a series of fractions of increasing molecular weight are obtained from the solutions [1]. A fractional extraction method was more efficient than the conventional precipitation fraction in obtaining fractions with a narrow MWD [37]. In fractional precipitation of biopolymers, separation of different polymers from mixtures in aqueous solutions, e.g. for proteins, may be achieved by variation of pH, iso-electric precipitation, by variation of ionic strength, salting-in and salting-out, or by the use of organic solvents, often ethanol, at low temperatures to prevent denaturation []. The procedure of SEC (GPC) is also a method of a polymer fractionation. A series of fractions may be collected from the effluent, with gradually increasing their molecular weights. The MWD may be calculated from the chromatograms [1].

3.4. Molecular Weight Distribution and Dispersity of Polymers

Some naturally occurring polymers such as certain proteins and nucleic acids consist of molecules with a specific molecular weight and are monodisperse [13,28]. Proteins are almost the only source of truly monodisperse polymers [38]. Nature makes all these molecules exactly alike [38]. Branching may occur, which broadens the MWD [28]. Other natural polymers, polysaccharides similar to most of synthetic polymers consist of molecules with different molecular weights and are polydisperse samples [13,28].

4. Conclusions

Determination of molecular weight distribution using various experimental procedures for polymers, synthetic and natural, with particular attention on biopolymers is reviewed. Nearly all synthetic polymers and some biopolymers are polydisperse and can be described in terms of one or more MWD functions. Among a different family of biopolymers, polysaccharides are polydisperse, whereas proteins, DNA and RNA are monodisperse. Chemical composition, siz or molecular weight of a homopolymer or a copolymer chain may vary. Two polymers may have exactly the same or similar average molecular weights but very different MWDs.

SEC is a reliable procedure for determination of the relative MWD. A series of standard polymer samples with definite molecular weights are required to determine molecular weight and MWD of unknown samples. The SEC method also makes possible a direct and simple determination of the resolution factor of the separation system employed.The combination of SEC with light scattering or another absolute method for molecular weight determination is an alternative solution for the quantitative evaluation of MWD and calculation of the DI, for polymers with both narrow and wide distributions.

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