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Large scale fractionation of pullulan and dextran

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Abstract

A recently developed large scale fractionation technique named continuous spin fractionation (CSF) was applied to fractionate pullulan and dextran. 450 g of pullulan with a broad molecular weight distribution were fractionated using water as solvent and acetone as precipitant. In this study, we have in five CSF runs prepared three fractions with apparent $\bar{M}_w^*$ values ranging from 17.6 to 413 kg mol^{-1}. Seventy grams of dextran were fractionated with a mixed solvent of water plus methanol. Five fractionation steps resulted in four samples with $\bar{M}_w$ values between 4.36 and 18.2 kg mol^{-1}.

Keywords: Dextran; Fractionation of polymers; Molecular weight distribution/molar mass distribution; Pullulan

1. Introduction

Pullulan and dextran are two of the most commonly used polysaccharides and find application in many fields like food science (Hiji, 1986; Ledward & In, 1979) health care (Nakashio, Tsuji, Toyota & Fujity, 1976), pharmacy (Childers, Oren & Seidler, 1991; Chourasia & Jain, 2004) and even lithography (Vermeersch, Coppens, Hauquier & Schacht, 1995). Due to its strictly linear structure pullulan is also very valuable in basic research as a well defined model substance (Adolphi & Kulicke, 1997; Arnosti, 1996). In many cases it is mandatory to have access to sufficiently large amounts of polysaccharide samples with well defined molecular weight distributions, e.g. for GPC-standards. This paper reports on experiments, which demonstrate for the first time that pullulan and dextran can be successfully fractionated on a large scale.

Because of the general demand for polymer samples with reasonable molecular and chemical uniformity we have recently developed a new fractionation method called continuous spin fractionation (CSF) (Eckelt, Haase, Loske, & Wolf, 2003; Eckelt et al., 2004). It overcomes some important limitations of continuous polymer fractionation (CPF) (Meißner & Wolf, 1998; Wolf, 1994), a precursor technique representing a particular form of counter current extraction. CSF solves above all problems with the damming back of the source phase and with the efficiency of the separation process at higher over-all polymer concentrations.

Both large scale fractionation methods are based on the liquid–liquid phase separation of polymer containing solutions (Flory, 1953). Phase separation is induced by mixing a homogenous polymer solution (feed, FD) with a liquid of sufficiently low solvent quality (extracting agent, EA). At the resulting overall composition (working point, WP) the system forms a polymer rich and a polymer lean phase. The former (gel phase) is very viscous and contains preferably the high molecular weight material of the initial polymer for enthalpic reasons. The latter phase (sol phase) is considerably less viscous and contains the shorter chain material because of entropic reasons. Due to the difference in their density, the two phases separate normally already upon standing. So far we have not observed differences between the two methods concerning the kinetics of macroscopic phase separation. In the rare cases where sedimentation is too slow, centrifugation can be used.

The variation of the process parameters (e.g. compositions of FD and EA, their mixing ratio and temperature) permits the control of the resultant average molar masses and the molecular weight distributions. A major feature of CSF consists in the use of spinning nozzles, similar to those employed in the fiber industry. The feed is pressed through

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their holes (diameters on the order of 50–100 μm) into the EA. Because of the Rayleigh instability (Rayleigh, 1879; Tomotika, 1936) the initially formed threads of feed break up into tiny droplets immediately after leaving the nozzle. This facilitates the transfer of the more soluble polymer species contained in this phase into the extracting agent to such an extent that successful fractionation becomes possible even with concentrated polymer solutions. Because of the comparatively high shear rates involved in the spinning process, one might expect chain scission under certain conditions. However, according to present experience the problem arises only with extremely high molecular weight material. For the systems under investigation gel permeation analysis has demonstrated the absence of flow induced polymer degradation.

2. Experimental part

2.1. Materials

For the present study we have used a sample called pullulan 200 k. The apparent molar masses are $\bar{M}^*_n = 79.7$ kg mol^{-1} and $\bar{M}^*_w = 277$ kg mol^{-1} resulting in a non-uniformity $U = (\bar{M}^*_w/\bar{M}^*_n) - 1 = 2.47$ according to GPC analysis. Dex10k, a relatively low molar mass sample of dextran, with $\bar{M}_w = 11.1$ kg mol^{-1} and $U = 1.01$ (according to GPC measurements) served as the starting material for the fractionation. Both polysaccharides were kindly donated by Polymer Standards Service (PSS), Mainz, Germany.

Deionized water was used as solvent for both polymers. Acetone (AC) and methanol (MeOH), puriss. Quality from Fluka, served as precipitant for pullulan and dextran, respectively, without further purification.

2.2. Methods

2.2.1. Gel permeation chromatography (GPC)

These experiments were carried out in aqueous solutions (containing 8.5 g $NaNO_3$ and 4.2 g $NaHCO_3$ per liter) using the columns HEMA BIO 40, HEMA BIO 1000, SUPREMA 300, supplied by PSS for GPC-measurements at room temperature. The differential refractometer Gynkotek RI-71 was employed as detector and dextran standards (PSS) served for calibration. The molar masss resulting for pullulan from GPC were obtained by means of universal calibration using the following Kuhn–Mark–Houwink parameters for pure water: $K_{Dextran} = 0.0978$ ml/g, $a_{Dextran} = 0.5$, $K_{Pullulan} = 0.0221$ ml/g and $a_{Pullulan} = 0.66$ (Kurata & Tsunashima, 1999). Because of the fact that our eluent contains salt, the obtained molar masses are only apparent values, as indicated by asterisks.

2.2.2. CSF apparatus

The starting polymer solution (feed) and the extracting agent were pumped with precision pumps (Ismatec BVP, ProMinent Gamma/5, allowing an accurate flux control) from their storage tanks into a mixing vessel under vigorous stirring. The feed exits through a spinning nozzle consisting of a gold/platinum alloy with 1035 holes of 60 μm diameter each. The two phase system arising from this procedure in the mixing vessel is pumped (Ismatec BVP pump) into a thermostatted container with a volume of 10 l, to allow for complete macroscopic phase separation.

2.2.3. Phase diagram

The information required for CSF was obtained in the usual manner (Schneider, Wünsch & Wolf, 2002). Cloud points were detected turbidimetrically by titrating homogeneous polymer solutions in an automated manner with the non-solvent, using equipment described earlier (Schneider et al., 2002). The critical polymer concentration was determined by means of phase volume ratios (Krause & Wolf, 1997). In order to obtain tie lines we have pressed homogenous polymer solution through a spinning nozzle into the extracting agent in such a manner, that the resulting over all composition lies within the miscibility gap and demixing into two liquid–liquid phases takes place. After macroscopic separation the coexisting phases were detached and analyzed with respect to their composition. To this end we collected the volatile components in a cooling trap and determined the composition of this mixture by means of refractometry. The remaining polymer was weighed.

The phase diagram of the H_2O/MeOH/Dextran is more complex because low molar mass dextran may crystallize from highly concentrated aqueous solution (Stenekes, Talsma & Hennink, 2001). For the fractionation this phenomena is of minor importance and the location of the solid–liquid phase boundary was, therefore, not determined.

3. Results and discussion

3.1. Pullulan

In order to find suitable mixed solvents for the fractionation of pullulan, we have tested several combinations of solvents and precipitants. Water was finally chosen as solvent, because of its availability and beneficial environmental properties. Acetone was selected as non-solvent on account of its adequate solvent power for fractionation, low boiling temperature (simplifying the polymer recovery) and large density difference to water (allowing fast macroscopic phase separation).

The phase diagram of the system water/acetone/pullulan, the basis for the CSF experiments performed, is presented in Fig. 1. This graph also shows the compositions of FD, EA, the WP resulting from the selected fluxes, and the composition of the two phases (Sol and Gel) obtained in the course of fractionation.

With the information gathered from preparatory experiments a fractionation strategy was established. As feed we

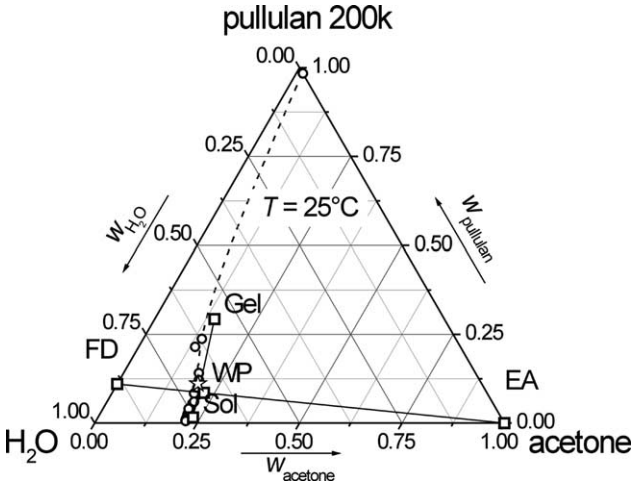

Fig. 1. Phase diagram of the system water/acetone/pullulan at 25 °C. Open circles indicate cloud points. The dashed line represents the probable continuation of the cloud point curve to the measured swelling point of pullulan in acetone. Also shown are the compositions of feed (FD), extracting agent (EA), of the working point (WP) and of the sol- and gel-phase of the first CSF-run. The open star marks the critical composition.

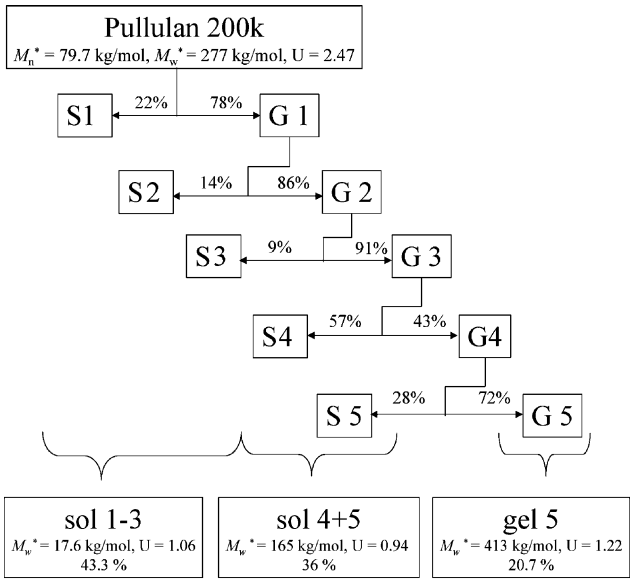

Fig. 2. Scheme for the fractionation of 450 g of pullulan 200 k. The percentage of the polymer contained in the feed recovered from the different phases is indicated. The sol fractions of the first three runs were combined to yield sol 1–3; the sol fractions of run 4 and 5 were also merged to sol 4 + 5. Weight average molar masses and non-uniformities of the final products are also indicated.

have chosen a binary mixture of water and pullulan and the target was to eliminate the lower molecular weight material of the polymer in the first fractionation steps. Normally, the selection of a ternary mixture close to the cloud point curve is recommended as feed, because of the reduction in the amount of solvent required. In this case we have not obeyed this rule because of the high room temperature of 35 °C prevailing in our laboratory during the summertime. Under these conditions the risk of changes in the composition of the feed due to evaporation of the highly volatile acetone would have been too high. Pure acetone was chosen as EA for the same reason.

In total we have performed five CSF runs, using the gel fraction of the previous CSF run in all cases as feed for the next run. Details of the fractionation strategy and its efficiency are presented in Fig. 2.

The first CSF run yielded a sol fraction that contained only the lowest molecular weight material of the pullulan. However, the gel fraction still contained significant amounts of the short chain material. For that reason the fractionation was repeated twice, using the resulting gel fractions as feed and working under similar conditions to remove most of the low molecular weight material. After three CSF-runs the sol fractions were combined due to their similar molar masses and non-uniformities. The remaining gel fraction was used as the starting material for another CSF-run.

The aim of the fourth CSF-run was to separate the very high molecular weight material components of pullulan. Thus, the solvent power was raised and the composition of the working point was moved towards lower pulluan concentrations (namely 2.3 wt%). This concentration is well below the critical polymer concentration of 11.1 wt% and only the longest chains remain in the gel fraction. The resulting high molecular weight sample was extracted once more, because it still contained too much lower molecular weight material. The two sol fractions where combined. After all, three fractions of different molar mass and non-uniformities markedly lower than the starting material could be achieved.

Fig. 3 shows the molecular weight distribution of the starting material and of the three final fractions. GPC analysis of the original pullulan reveals a trimodal distribution (as with some other biopolymers like starch and its derivatives (Gosch, Haase, Kulicke & Wolf, 2002)).

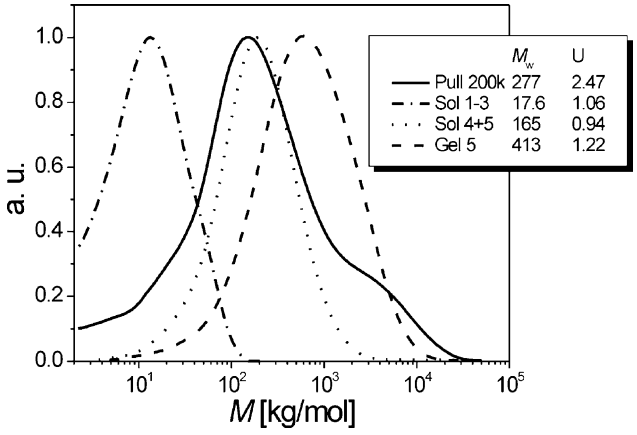

Fig. 3. Molecular weight distributions (normalized to the same height of the maximum) of the initial pullulan sample and of the three fractions obtained. The weight average molar mass in kg/mol and the non-uniformities are indicated in the graph.

Due to successful fractionation the distribution of the final samples is unimodal. From the insert in this graph one can also see that the non-uniformity of the starting material could be reduced considerably from 2.47 to 1.06, respectively, 0.94 with the sol factions and to 1.22 with the final gel fraction. For the assessment of the results it is worthwhile to note that the fractionation efficiency of CSF is normally considerably higher than that of CPF performed with the same system under else identical conditions (Eckelt & Wolf, in press).

3.2. Dextran

For this hydrocarbon water was chosen as solvent and methanol as precipitant. According to orienting experiments acetone had to be ruled out as non-solvent because of its lower fractionation power and tetrahydrofuran constitutes too weak a non-solvent. The phase diagram of the system water/methanol/dextran is given in Fig. 4. It shows cloud points and the compositions of FD, EA, and WP (resulting from the selected fluxes of the first CSF-run). No swelling point was determined for dextran in methanol, because of the difficulties to prepare thin films; due to the crystallization tendency of the present polymer sample the resulting films are so brittle that they break immediately.

During macroscopic phase separation the dextran contained in the gel phase starts (because of its high polymer content) to crystallize, while the polymer that is present in the sol phase remains in solution. For fractionation this feature does not represent a problem because it takes typically 1 h for crystallization to set in. To regain the partially crystalline dextran of the gel phase for the next fractionation step it was redissolved in boiling water for about 2 h and then freeze dried. Fig. 5 shows the five fractionation steps that have been performed so far.

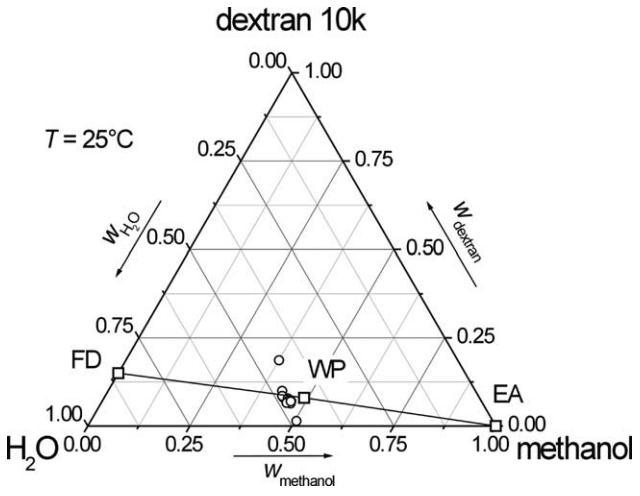

Fig. 4. Phase diagram of the system water/acetone/dextran at 25 °C. Open circles indicate cloud points. Also shown are the compositions of feed (FD), extracting agent (EA) and working point (WP) of the first CSF-run.

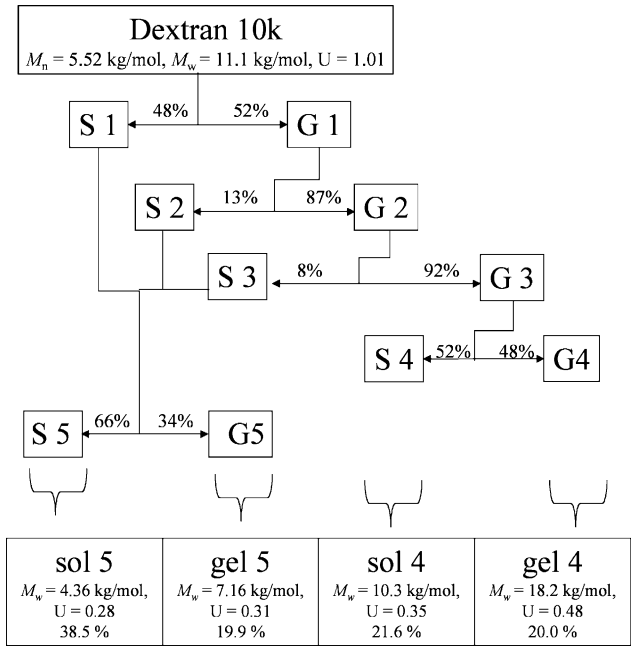

Fig. 5. Scheme for the fractionation of 70 g of dextran 10 k. The percentage of the polymer contained in the feed that was found in the different phases is indicated. The sol fractions of the first three runs were combined and fractionated to yield sol 5 and gel 5; the gel fraction of run 3 was fractionated to result in sol 4 and gel 4. Also given are weight average molar masses and non-uniformities of the final products.

In the first CSF-run nearly the same fraction of the starting material was found in the two phases. The polymer in the gel phase still contained considerable amounts of low molecular weight material. In order to take out the remaining short chain dextran this CSF run was repeated twice under the same conditions, using the previous gel fraction as starting material. The amount of polymer that can be found in the sol phase for the second and third CSF-run decreases because the miscibility gap expands (higher average molar mass of the respective feed) and the working point is now located deeper inside the two phase region. In the third CSF-run only 8 wt% of the starting material was extracted into the sol phase. Like with pullulan the sol fractions of the first three CSF-runs where combined due to their similar molecular weight distributions.

For the fourth CSF-run, which uses the gel fraction of the third run as starting material, the polymer concentration of the working point was again reduced, this time to 0.84 wt%, in order to transfer more polymer material into the sol phase and to produce a gel fraction that contains only the longest chains of the initial sample. In a fifths CSF experiment we have removed the lower molecular weight components from the merged sol fractions 1–3 at a total polymer concentration of 2 wt%.

Fig. 6 shows the molecular weight distribution of the starting material and the four final fractions. The insert in

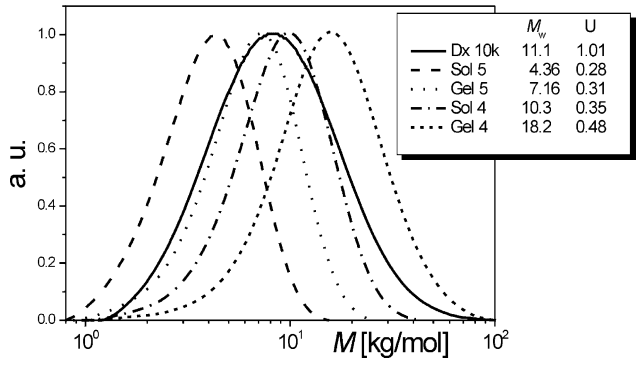

Fig. 6. Molecular weight distributions of the initial dextran sample and of the four obtained fractions normalized to the same height. Weight average molar masses and non-uniformities are indicated in the graph.

the graph gives the molar masses and the non-uniformity of the different samples showing that the molecular weight distribution has been significantly narrowed. From the fact that the molecular weight distribution of the different fractions can be summed up to the initial distribution it can be concluded that the polymer did neither degrade during fractionation nor during its recovery.

4. Outlook

The present study demonstrates that pullulan and dextran can be fractionated in large amounts with reasonable expenditure by means of continuous spin fractionation using the mixed solvents water/acetone and water/methanol, respectively. So far the non-uniformities of the thus obtained samples may still not be low enough for some purposes. This, however, is no principal shortcoming of the method, because additional CSF runs offer the possibility to reduce the non-uniformity further. In this context, it appears worthwhile to note that the expenditure in labor and solvents increases considerably if one aims at samples with $U < 0.2$. Fortuitously, the requirements concerning molecular uniformity are normally not so stringent even for high duty polymers. In many cases it suffices to remove either adverse low or high material.

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