



# Effect of polymer molecular weight distribution on solute sequestration in two-phase partitioning bioreactors



Stuart L. Bacon, Andrew J. Daugulis, J. Scott Parent\*

Department of Chemical Engineering, Queen's University, 19 Division St., Kingston, Ontario K7L 3N6, Canada

## HIGHLIGHTS

- Solute PC was a predictable function of a polymer's number average molecular weight ( $M_n$ ).
- PC was independent of molecular weight distribution (MWD) (e.g. unimodal, bimodal).
- Polymer complex modulus ( $G^*$ ) was dependent on both  $M_n$  and MWD.
- Bimodal polymer formulations had higher PCs compared to unimodal materials at equivalent  $G^*$ .
- Mixtures of an organic solvent and high MW polymer exhibited further PC improvements.

## ARTICLE INFO

### Article history:

Received 18 February 2016

Received in revised form 7 April 2016

Accepted 8 April 2016

Available online 16 April 2016

### Keywords:

Biodegradation

Bioprocess

Polymer thermodynamics

Absorption

Extractive fermentation

## ABSTRACT

Polymeric solids are effective absorbents in two-phase partitioning bioreactors (TPPBs) when they provide adequate absorptive capacity for the target solute, as well as the physical properties required by solid–liquid TPPB operations. This study demonstrates the influence of molecular weight distribution (MWD) on solute uptake, as measured by solute partition coefficient (PC), and mechanical strength, as measured by the polymer's complex modulus ( $G^*$ ). Experimental PC data for *n*-octanol absorption from aqueous solution by poly(dimethyl siloxane) (PDMS) demonstrate a decline in absorptive capacity with increasing number average molecular weight ( $M_n$ ), in agreement with Flory–Huggins solution theory predictions. Importantly, MWD is shown to have no effect on solute uptake, with both unimodal and bimodal distributions generating the same PC at a given  $M_n$ . This is in contrast to  $G^*$ , whose MWD sensitivity is exploited to formulate bimodal mixtures of poly(isobutylene) (PIB) that provide high *n*-octanol PC values as well as satisfactory material strength. This bimodal MWD strategy to TPPB absorbent design is extended to miscible solutions of high MW PIB and cyclohexylbenzene, which are shown to generate superior *n*-octanol PC at a given  $G^*$ .

© 2016 Elsevier B.V. All rights reserved.

## 1. Introduction

Two-phase partitioning bioreactor (TPPB) technology is a proven means of improving bioreactor productivity by reducing substrate and/or product cytotoxicity. The first TPPB processes employed an organic solvent [1–5] or oligomeric liquid (e.g. silicone oil, poly(propylene glycol)) [6–11] as an immiscible second phase to selectively sequester inhibitory compounds from fermentation media. However, low molecular weight absorbents can suffer from biocompatibility and bioavailability issues, as well as operational challenges stemming from volatility, flammability, emulsification and foaming [1,12–17]. Viscoelastic polymers are

a potential solution to these challenges [18–20], provided they possess the necessary absorptive capacity, and the physical integrity needed to survive within a bioreactor environment.

A principal polymer selection criterion is thermodynamic affinity for the target solute, as quantified by the partition coefficient (PC) between the polymer and an aqueous phase. The influence of chemical structure, crystallinity and phase transition temperatures on PC has been investigated in detail [21,22], as has the effect of these polymer characteristics on solute diffusivity and mass transfer rate [19,23–30]. The present work advances our understanding of the effect of polymer molecular weight distribution (MWD) on solute absorption. Our preliminary studies have shown that lowering the MW of poly(*n*-butyl acrylate) [21] and poly(propylene glycol) [9] has a positive effect on PC, presumably due to associated increases in the entropy of mixing [31]. These examples involved unimodal MWD's of linear polymer chains,

\* Corresponding author.

E-mail address: [scott.parent@queensu.ca](mailto:scott.parent@queensu.ca) (J.S. Parent).

leaving the potential advantages provided by other polymer architectures largely unexplored in the context of TPPB applications.

The central question addressed by this study is whether unimodal MWD polymers and bimodal MWD polymers differ appreciably in terms of solute uptake and material strength. We begin with PC measurements of *n*-octanol absorption by poly(dimethyl siloxane) (PDMS, silicone oil) samples of varying molecular weight and MWD. Both substances are well represented in the bioprocessing literature, with *n*-octanol forming the basis of tabulated  $\log K_{o/w}$  data, and silicone oil finding frequent application in TPPB systems [6–8,10,11,32]. These data are discussed in the context of Flory–Huggins solution theory to provide a thermodynamic basis for observed MW effects.

The joint influence of MWD on solute uptake and material strength is examined using poly(isobutylene) (PIB), which provides the mechanical strength that is generally lacking in uncured PDMS materials. Measurements of *n*-octanol PC and complex modulus ( $G^*$ ) reveal important differences between unimodal and bimodal MWD samples of linear PIB chains. Insights gained through these studies are used to formulate mixtures of high MW PIB with a high-boiling organic solvent, cyclohexylbenzene, which are designed to exploit the absorptive capacity of a low MW compound as well as the physical strength of a high MW polymer. The results are relevant to TPPB bioproduction/biodegradation processes and polymer-based product recovery processes that require solute affinity and mechanical rigidity, such as pervaporation, pertraction and vapor permeation.

## 2. Materials and methods

### 2.1. Materials and material preparation

All chemicals (purity  $\geq 98\%$ ) were purchased from either Sigma–Aldrich (Canada) or Fisher Scientific (Canada). All poly(dimethyl siloxane) (PDMS) samples (S-UM-1 to S-UM-5) were purchased from Scientific Polymer Products (Ontario, NY). PIB samples IB-UM-1 to IB-UM-4 were provided by TPC group (Houston, TX). PIB samples IB-UM-5 and IB-UM-6 were provided by Shandong Hongrui Petrochemical Co., Ltd (Jinan, China). Samples IB-UM-7 to IB-UM-9 were purchased from Scientific Polymer Products (Ontario, NY). Number average ( $M_n$ ) and weight average ( $M_w$ ) molecular weight data for each polymer are listed in Tables 1 and 2, experimentally determined as per Section 2.2.

Bimodal PDMS samples were prepared by adding varying quantities of S-UM-1 to S-UM-5 and agitated in an Innova 4400

**Table 1**

$M_n$  and  $M_w$  values for unimodal PDMS samples (S-UM) and bimodal mixtures of high MW PDMS mixed with oligomeric PDMS (S-BM). S-BM samples were made by combining S-UM-1 and S-UM-5.

Sample ID	Low MW component	Low MW fraction	$M_n$ (g/mol)	$M_w$ (g/mol)
S-UM-1	n/a <sup>a</sup>	0	100 <sup>b</sup>	105 <sup>b</sup>
S-UM-2	n/a <sup>a</sup>	0	1150	1,200
S-UM-3	n/a <sup>a</sup>	0	9220	15,500
S-UM-4	n/a <sup>a</sup>	0	21,600	48,400
S-UM-5	n/a <sup>a</sup>	0	69,700	135,300
S-BM-1	PDMS oligomer	0.95	105	6580
S-BM-2	PDMS oligomer	0.90	110	13,300
S-BM-3	PDMS oligomer	0.75	130	34,400
S-BM-4	PDMS oligomer	0.50	200	67,700
S-BM-5	PDMS oligomer	0.25	400	101,500
S-BM-6	PDMS oligomer	0.10	980	121,800
S-BM-7	PDMS oligomer	0.05	1880	128,300

<sup>a</sup> Unimodal sample.

<sup>b</sup> Nominal value from supplier.

**Table 2**

$M_n$  and  $M_w$  values for unimodal PIB samples (IB-UM), bimodal mixtures of high MW PIB with oligomeric PIB (IB-BM) and bimodal mixtures of high MW PIB with CHB (IB-CHB). IB-BM samples were made by combining IB-UM-1 and IB-UM-9. IB-CHB samples were made by combining IB-UM-9 with CHB.

Sample ID	Low MW component	Low MW fraction	$M_n$ (g/mol)	$M_w$ (g/mol)
IB-UM-1	n/a <sup>a</sup>	0	670	1100
IB-UM-2	n/a <sup>a</sup>	0	920	1790
IB-UM-3	n/a <sup>a</sup>	0	1470	3110
IB-UM-4	n/a <sup>a</sup>	0	2670	8490
IB-UM-5	n/a <sup>a</sup>	0	9490	51,400
IB-UM-6	n/a <sup>a</sup>	0	18,400	65,500
IB-UM-7	n/a <sup>a</sup>	0	21,200	119,800
IB-UM-8	n/a <sup>a</sup>	0	85,700	503,800
IB-UM-9	n/a <sup>a</sup>	0	320,000	4,912,000
IB-BM-1	PIB oligomer	0.75	890	1,229,000
IB-BM-2	PIB oligomer	0.50	1320	2,446,000
IB-BM-3	PIB oligomer	0.25	2640	3,677,000
IB-BM-4	PIB oligomer	0.10	6490	4,416,000
IB-BM-5	PIB oligomer	0.05	12,500	4,659,000
IB-BM-6	PIB oligomer	0.01	50,400	4,857,000
IB-CHB-1	Cyclohexylbenzene	0.25	640	3,684,000
IB-CHB-2	Cyclohexylbenzene	0.11	1520	4,396,000
IB-CHB-3	Cyclohexylbenzene	0.04	3860	4,710,000
IB-CHB-4	Cyclohexylbenzene	0.02	9160	4,828,000

<sup>a</sup> Unimodal sample.

incubator shaker at 30 °C and 180 rpm until the solution was homogeneous (one week). The solution was vortexed at least daily under high power to facilitate homogeneity. Bimodal PIB mixtures (IB-BM) were prepared by dissolving varying quantities of IB-UM-1 and IB-UM-9 as a ~15 wt% solution in hexanes in a sealed glass tube. The resultant polymer cement was agitated at 180 rpm in an Innova 4400 incubator shaker at 60 °C until the solution was homogeneous (one week). The solution was vortexed at least daily under high power to facilitate homogeneity. Aliquots of the polymer mixtures were transferred to scintillation vials and allowed to dry at 60 °C for one week followed by atmospheric conditions for one additional week.

Bimodal mixtures of high MW PIB and cyclohexylbenzene (IB-CHB) were prepared by adding varying quantities of cyclohexylbenzene (CHB) to IB-UM-9 and agitated in an Innova 4400 incubator shaker at 30 °C and 180 rpm until the solution was homogeneous (two weeks). The solution was vortexed at least daily under high power to facilitate homogeneity. The rationale for selecting CHB is provided in considerable detail in Section 3.4. Low molecular weight component fractions (wt/wt) were calculated by determining the weight loss from a sample after drying in a 110 °C oven.

### 2.2. Molecular weight determination

PIB and PDMS samples with  $M_n < 100,000$  g/mol were characterized by size exclusion chromatography (SEC) using a Waters 2960 separation module with Styragel packed columns HR 0.5, HR 1, HR 3, HR 4, and HR 5E (Waters Division Millipore) coupled with a refractive index (RI) detector operating at 35 °C. Tetrahydrofuran (THF) was used as eluent and the flow rate was set to 1.0 mL/min.

PIB samples with  $M_n > 100,000$  g/mol were characterized by SEC using a Viscotek 270max separation module with an RI detector. A set of two porous PolyAnalytik columns with an exclusion limit molecular weight of  $20 \times 10^6$  g/mol were used in series at 40 °C. Distilled THF was used as the eluent at a flow rate of 1.0 mL/min.

In both cases, the weight average molecular weight ( $M_w$ ), number average molecular weight ( $M_n$ ) and polydispersity index (PDI)

were obtained by universal calibration using Mark–Houwink parameters [33].

$M_n$  and  $M_w$  values for the homogenous bimodal mixtures were calculated using the following equations:

$$M_{n,\text{mix}} = \frac{1}{\frac{w_{lo}}{M_{n,lo}} + \frac{w_{hi}}{M_{n,hi}}} \quad (1)$$

$$M_{w,\text{mix}} = w_{lo}M_{w,lo} + w_{hi}M_{w,hi} \quad (2)$$

$$w_{lo} = 1 - w_{hi} \quad (3)$$

where  $w_i$  is the weight fraction of component 'i' in the mixture.

### 2.3. Experimental partition coefficients ( $PC_{\text{expt}}$ )

Partition coefficient tests were performed in triplicate using three polymer masses, ranging from 0.25 to 1.5 g. *n*-Octanol was prepared in aqueous solution (0.4 g/L) using Type I ultrapure water. In addition to the polymer mass, a 20 mL aliquot of aqueous solution were added to each scintillation vial, sealed tightly with a foil lined cap and allowed to equilibrate in an Innova 4400 incubator shaker at 30 °C at 180 rpm for 1 week (PDMS) or 2 weeks (PIB). Equilibration times were determined from time-course absorption experiments for each material. *n*-Octanol concentrations before and after equilibration with the polymer were measured using a Varian 450-GC gas chromatography unit equipped with a CP-8410 AutoInjector, VF-5 ms 30 m capillary column and FID detector. Using this method, 0.4 g/L control *n*-octanol solutions (left to equilibrate without polymer) had a standard deviation of 6.7% ( $n = 3$ ).

A mass balance was performed to determine the solute concentration in the polymer. Experimental PC values were calculated using aqueous and polymer phase weight fractions,  $w_i^{\text{aq}}$  and  $w_i^{\text{poly}}$ , in Eq. (4). Standard deviation ( $n = 3$ ) values were calculated from triplicate samples to establish a mean value for the equilibrium PC.

$$PC = \frac{w_i^{\text{poly}}}{w_i^{\text{aq}}} \quad (4)$$

### 2.4. Partition coefficients predictions ( $PC_{\text{pred}}$ )

Partition coefficients (PC) can be predicted for systems only at thermodynamic equilibrium, for which the PC has been defined as [21]

$$PC = \frac{w_i^{\text{poly}}}{w_i^{\text{aq}}} = \frac{\Omega_i^{\text{aq}}}{\Omega_i^{\text{poly}}} \quad (5)$$

In this study, the non-random two liquid (NRTL) model (Eq. (6)) [34] was selected to estimate aqueous phase weight fraction activity coefficients ( $\Omega_i^{\text{aq}}$ ) at experimental conditions.

$$\begin{aligned} a_i^{\text{aq}} &= w_i \Omega_i^{\text{aq}} = x_i \gamma_i^{\text{aq}} \\ &= x_i \exp \left( x_2^2 \left( \tau_{21} \frac{\exp(-2\alpha_{12} \tau_{21})}{[x_1 + x_2 \exp(-\alpha_{12} \tau_{21})]^2} + \tau_{12} \frac{\exp(-\alpha_{12} \tau_{12})}{[x_2 + x_1 \exp(-\alpha_{12} \tau_{12})]^2} \right) \right) \end{aligned} \quad (6)$$

where  $a_i^{\text{aq}}$  is the activity of *n*-octanol in the aqueous phase,  $\gamma_i^{\text{aq}}$  is the mole fraction activity coefficient and  $x_i$  is the mole fraction of 'i'. Temperature dependent LLE binary interaction parameters were provided by the Dortmund Data Bank [35] for *n*-octanol (1)/water (2):  $\alpha_{12} = \alpha_{21} = 0.2$ ;  $\Delta g_{12}[\text{K}] = -3902.9 + 24.68 \cdot T - 0.0388 \cdot T^2$ ;  $\Delta g_{21}[\text{K}] = 2099.1 - 0.316 \cdot T + 0.00882 \cdot T^2$ . Note that according to DDB convention  $\tau_{ij} = \frac{\Delta g_{ij}}{T}$  and  $G_{ij} = \exp(-\alpha \cdot \tau_{ij})$ .

As described in our previous work [21,22], polymer phase activity coefficients ( $\Omega_i^{\text{poly}}$ ) were predicted using the Flory–Huggins

solution theory [31] in conjunction with Hansen solubility parameters (FH–HSP) [36]. The system-dependent adjustable parameter in the FH–HSP model was set to a default value of  $\alpha = 1$  [21,22,36]. Solubility parameters were sourced from the database provided in the HSPiP v.4.0.04 computer program [37]. Octanol [ $\text{MPa}^{1/2}$ ]: ( $\delta_D$ ,  $\delta_P$ ,  $\delta_H$ : 16, 5, 11.2) and PDMS [ $\text{MPa}^{1/2}$ ]: ( $\delta_D$ ,  $\delta_P$ ,  $\delta_H$ : 12.3, 0.4, 0.3).

### 2.5. Rheological analysis

Polymer samples were tested for complex modulus  $G^*$  at 37 °C in an Advanced Polymer Analyzer 2000 (Alpha Technologies) controlled-strain rheometer equipped with biconical disks operating at 1 Hz and 3° arc.

## 3. Results and discussion

### 3.1. Unimodal and bimodal MWD absorbents

Polymeric materials, either by design or inherent from their method of preparation, do not have a single molecular weight (MW) in the manner of organic solvents, but a distribution of MWs that can be tailored to satisfy different physical and chemical property requirements. In the majority of cases, the complete MWD is not specified, but rather is summarized by the first two moments of the distribution. The number average MW,  $M_n$ , is calculated based on the number of each chain of a given molecular weight, while the weight average MW,  $M_w$ , is calculated on the mass of each chain of a given molecular weight. The ratio of  $M_w$  to  $M_n$  is the polydispersity,  $PD = M_w/M_n$ , a measure of the breadth of the distribution ranging from a value of 1 for a completely monodisperse material to very large values for materials possessing chains of widely ranging MWs.

The critical molecular weight of a polymer ( $M_c$ ), which defines the MW at which chain entanglements contribute to physical properties, is particularly important to this work. Below  $M_c$ , material viscosity is dominated by intermolecular forces, whereas above  $M_c$ , polymer chains entangle with one another to produce the viscoelasticity associated with elastomeric behavior. The polymer samples used in this study spanned a very wide range of MWs, as illustrated in Fig. 1a for “as received” PIB materials, and in Fig. 1b for bimodal PIB samples prepared by mixing different proportions of PIB oligomer and high MW PIB polymer. Taken together, these unimodal and bimodal samples shed light on the influence of MW and MWD on solute uptake and material physical properties, leading to a better understanding of how polymeric absorbents can be designed to meet the demands of TPPB systems.

### 3.2. Influence of PDMS $M_n$ , $M_w$ and MWD on *n*-octanol absorption

The selection of an absorptive phase for TPPB applications is normally concerned with chemical composition. Generalized statements such as “like dissolves like”, and empirical frameworks such as the Hildebrand solubility parameter [38] and the Hansen parameters [39] are expressions of the need to minimize the enthalpy of mixing generated by a prospective solute/solvent system. Semi-empirical expressions for the thermodynamic activity of a solute in a polymer phase, such as the Flory–Huggins solution model, also address the enthalpic contributions to the Gibbs energy directly through binary interaction parameters estimated from experimental data [31,36]. Note, however, that the Flory–Huggins equation has additional terms describing the entropic contributions to the Gibbs energy of mixing, wherein the molecular weight of the polymer phase factors prominently.

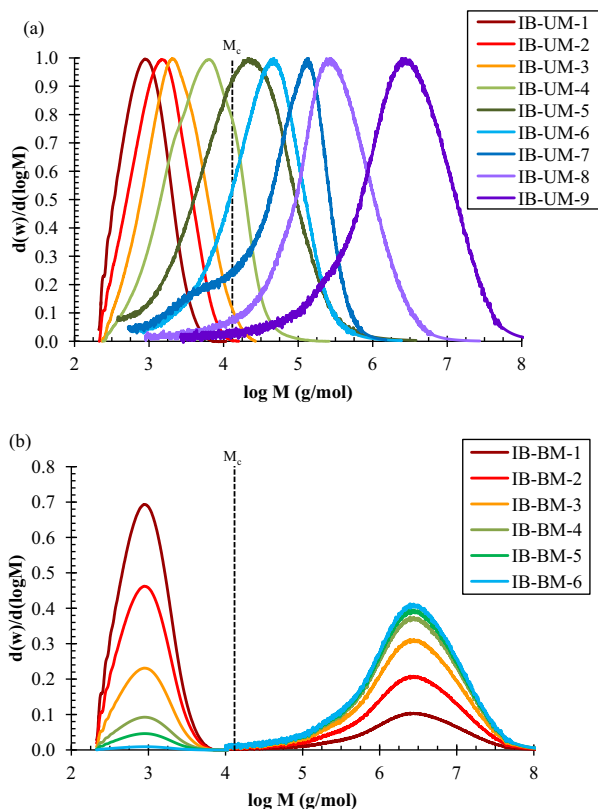


Fig. 1. Normalized GPC curves of (a) unimodal and (b) bimodal PIB samples.

Eq. (7) is a common presentation of the Flory–Huggins equation governing the activity coefficient of solute  $i$  in polymer  $j$ ;

$$a_i^{\text{poly}} = w_i \Omega_i^{\text{poly}} = x_i \gamma_i^{\text{poly}} = x_i \cdot \exp \left( \underbrace{\ln \frac{\varphi_i}{x_i} + 1 - \frac{\varphi_i}{x_i}}_{\text{combinatorial}} + \underbrace{\chi_{ij} \varphi_j^2}_{\text{non-comb}} \right) \quad (7)$$

where  $\gamma_i$  is the mole fraction activity coefficient,  $x_i$  is the mole fraction and  $\varphi_i$  is the volume fraction. Non-combinatorial (enthalpic) contributions to mixing are captured by the Flory–Huggins interaction parameter,  $\chi_{ij}$  [40], while combinatorial (entropic) contributions are a function of the volume fraction and the mole fraction of the solute in the mixture – the latter being directly related to the molecular weight of the polymer [31,36].

Fig. 2 provides a semi-log plot of predicted  $n$ -octanol PC values in PDMS derived from Flory–Huggins solution theory (See Section 2.4), as well as experimental PC data for our unimodal MWD and bimodal MWD samples. The data show that decreasing the  $M_n$  of PDMS from 69,700 to 100 g/mol results in a 2-fold increase in PC. According to the Flory–Huggins equation, decreasing polymer  $M_n$  lowers the solute mole fraction ( $x_i$ ) relative to solute volume fraction ( $\varphi_i$ ). Accordingly, the net contribution to solute activity ( $a_i$ ) is negative, indicating more favorable mixing and improved PC. At  $M_n$  values above about 1000 g/mol, the combinatorial contribution becomes negligible compared to the enthalpic term, resulting in a plateau for PC that agrees well with experiment. Inaccuracy in measuring the MW of PDMS oligomers may account for some of the deviation between model and experiment at low  $M_n$ .

The results presented in Fig. 2 have two important consequences for selecting polymers for TPPB applications. First, the influence that polymer MW has on PC is a predictable function of  $M_n$ , so the reporting of PC data, and their use in polymer selection,

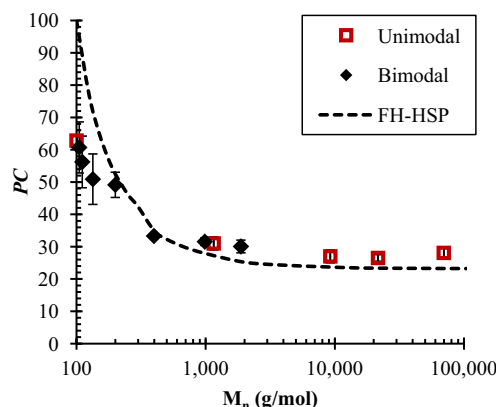


Fig. 2.  $n$ -Octanol partition coefficient versus  $M_n$  for unimodal (S-UM) and bimodal (S-BM) PDMS.

should make explicit reference to number average molecular weight, particularly at low  $M_n$ . Second, the influence of  $M_n$  on PC is independent of molecular weight distribution, applying equally well to unimodal and bimodal MWDs. Therefore, given the sensitivity of most physical properties to MWD and chain architecture, polymeric absorbents can be formulated to meet a PC/ $M_n$  target while simultaneously meeting mechanical strength targets. This opportunity is demonstrated below for the PIB polymer system, which provides superior mechanical strength to uncured PDMS.

### 3.3. Influence of PIB MWD on octanol uptake and material strength

As discussed above, the selection of a polymeric absorbent for TPPB applications requires consideration of its thermodynamic affinity for the target solute and its mechanical strength/toughness. Given the wide range of MWs of our samples and the resulting breadth of their physical properties, oscillatory rheometry was used as a standard measure of material strength. The complex modulus ( $G^*$ ) is the magnitude of the dynamic stress response by the polymer to an oscillatory applied strain. In contrast to a continuous strain rate measurement such as static tensile testing, it provides accurate data for oligomeric materials (viscous liquids) as well as high MW polymers (viscoelastic solids). Since PDMS requires crosslinking to infinite molecular weight to exhibit solid-like mechanical properties, it is not appropriate for studies of  $M_n/PC$  and  $M_n/G^*$  relationships. PIB was, therefore, selected for use in the remainder of the work.

The data plotted in Fig. 3 confirm that  $n$ -octanol partitioning into an amorphous elastomer improves as polymer  $M_n$  is lowered, as PC values for PIB increased nearly 4-fold ( $10.7 \pm 1.3$  to  $39.5 \pm 0.6$ ) as  $M_n$  decreased from 320,000 to 670 g/mol. Furthermore, the MWD of the material had no bearing on solute uptake capacity (PC), consistent with our PDMS/ $n$ -octanol results. Note that the end-groups of our PIB and PDMS samples were chemically similar to the polymer backbone. However, when the polarity and H-bonding strength of end-groups differ significantly from those of the main chain, the relationship between PC and oligomer MW can be dominated by enthalpic contributions as opposed to entropic effects, leading to deviations from Flory–Huggins solution theory on solute absorption in low MW oligomers has been demonstrated previously [41].

Our PDMS and PIB results showed that two chemically identical samples with a given  $M_n$  will provide the same PC, irrespective of MWD. In sharp contrast, the data plotted in Fig. 4 demonstrate the sensitivity of  $G^*$  to chain populations larger than the critical MW of the polymer ( $M_c$ ). The unimodal MWD samples generated

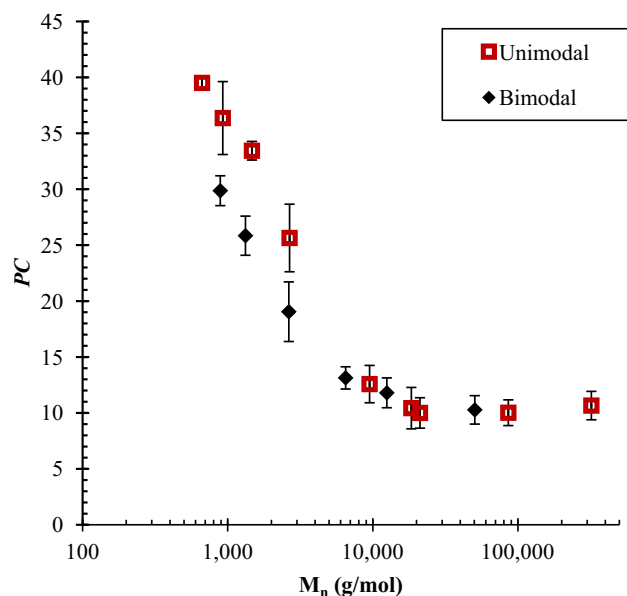


Fig. 3. Partition coefficient (PC) values plotted against (a)  $M_n$  and (b)  $M_w$  for unimodal and bimodal PIB.

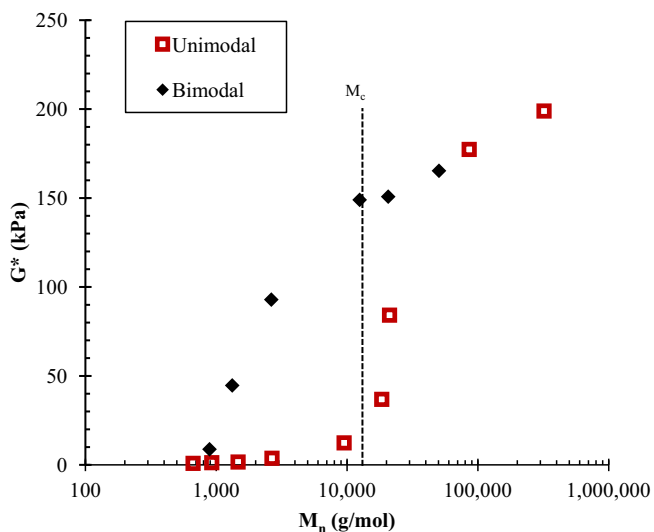


Fig. 4. Effect of  $M_n$  on complex modulus ( $G^*$ ) for unimodal and bimodal MWD PIB materials.

very low  $G^*$  values until a fraction of PIB chains had MW greater than  $M_c = 13,100$  g/mol [42,43], whereupon the material strength improved greatly as polymer chain entanglement heightened the material's resistance to an applied deformation [44,45]. Very different behavior was observed for the bimodal MWD samples that were prepared by mixing low MW PIB with high MW PIB (see Fig. 1). Since each bimodal mixture contained a population of chains larger than  $M_c$ , the bimodal materials provided greater  $G^*$  values at a given  $M_n$  than the unimodal materials. Consider that at  $M_n \sim 2650$  g/mol, the bimodal mixture generated a  $G^*$  25-times that of the corresponding unimodal material (Fig. 4).

In the context of TPPB polymer selection, it is clear that thermodynamic affinity for the target solute (PC) encourages the selection of low  $M_n$  elastomer, while mechanical strength ( $G^*$ ) favors high  $M_n$  material. Fortunately, compromises between PC and  $G^*$  that are inherent to conventional unimodal MWD polymers can be mitigated using bimodal MWD materials made from mixing very

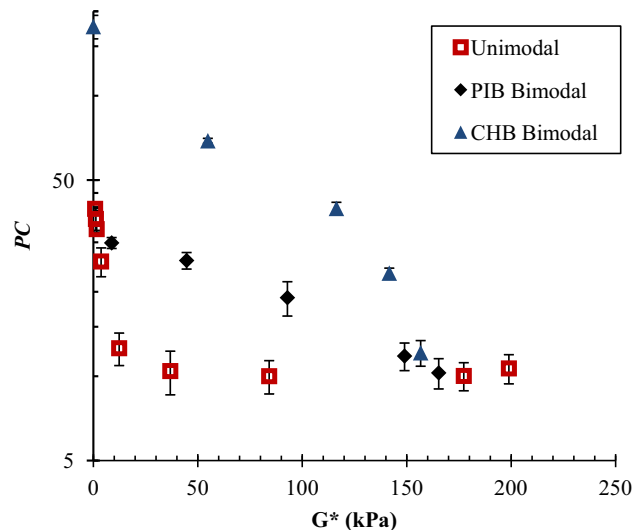


Fig. 5. Comparison of PC and  $G^*$  for unimodal PIB, bimodal PIB oligomer + PIB polymer, and bimodal CHB + PIB polymer samples.

low and very high MW material. Semi-log plots of PC versus  $G^*$  (Fig. 5) confirm that a bimodal mixture can combine much of the absorptive benefits derived from a small  $M_n$  with the strength derived from a polymer chain population with MW  $> M_c$ . In TPPB applications where liquid-like materials exhibit operational challenges such as foaming and emulsification, bimodal molecular weight distributions may provide desirable viscoelastic solid properties without incurring significant losses of solute absorptive capacity. Note that further benefits may be realized with a high MW component that contains long-chain branching, wherein chain entanglements effects are further amplified.

#### 3.4. Formulation of polymer + solvent absorbents

The benefits of bimodal MWD materials outlined above can be used to design interesting TPPB absorbents from miscible mixtures of a low MW organic solvent and high MW polymer. According to this approach, the polymer's primary role in the mixture is to provide the requisite material strength to mitigate operational challenges (e.g. foaming, emulsification) associated with pure organic solvents. The solvent is then selected to maximize the solute PC through both enthalpic and entropic contributions. Building upon our knowledge of the *n*-octanol/PIB system, we sought an organic solvent to serve as the low MW component for bimodal samples that provide a more favorable PC versus  $G^*$  relationship than that generated by combining low and high MW PIB alone. The development of a two-component formulation introduces additional material selection criteria, a list of which can be found in Table 3. Note that an organic solvent within

Table 3  
Ideal properties of a low molecular weight component in bimodal MWD absorbents.

Property	Selection criterion
Affinity for the target molecule	High <i>n</i> -octanol PC
Miscibility with polymer	Hansen solubility parameters
Low volatility	Boiling point/vapor pressure
Biocompatibility	Critical $\log K_{o/w}$ of microorganism
Must not leach into bioreactor broth preventing losses and causing cytotoxic effects	Water solubility
Commercial availability and cost	

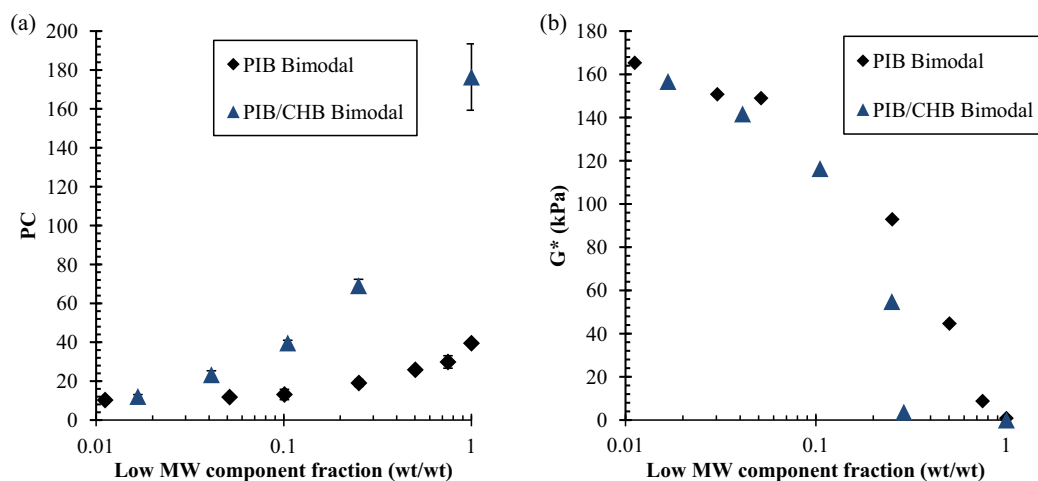


Fig. 6. Effect of low MW component fraction (oligomeric PIB or cyclohexylbenzene) on the absorption of *n*-octanol (PC) and polymer mixture stiffness  $G^*$ .

polymer solution will exhibit a reduced volatility and water solubility compared to the solvent alone.

Cyclohexylbenzene (CHB) was chosen on the basis of its a high boiling point ( $B_p = 239\text{--}240\text{ }^\circ\text{C}$ ) [46], low vapor pressure (6.1 Pa at  $25\text{ }^\circ\text{C}$ ), low water solubility (11.3 mg/L at  $25\text{ }^\circ\text{C}$ ), as well as a  $\log K_{o/w}$  (4.8) high enough to ensure biocompatibility with most industrially-pertinent microorganisms [10,15,47]. A predicted PC value of 212 for *n*-octanol was generated using the UNIFAC–vdW–FV model [48,49], which was later supplemented with an experimental value of  $PC_{\text{expt}} = 176 \pm 17$ . Note that this is considerably greater than the PC generated by oligomeric PIB ( $M_n = 670$ ,  $PC = 39.5 \pm 0.6$ ).

High MW PIB ( $M_n = 320,000$ ) was mixed in different proportions with either CHB or oligomeric PIB ( $M_n = 670$ ) to generate the materials whose properties are illustrated in Fig. 6. At all concentrations, samples containing CHB as the low MW component yielded higher *n*-octanol PCs than the corresponding oligomeric PIB containing controls, with the greatest gains corresponding to the highest loadings of low MW component (Fig. 6a). The effect of the low molecular component (CHB or oligomeric PIB) on  $G^*$  was similar at low concentrations (Fig. 6b). However, above a low MW component fraction of 0.2 (wt/wt), declines of  $G^*$  were steeper for the CHB loaded material, owing to the lower viscosity of the organic solvent relative to the PIB oligomer.

A summary of the PC versus  $G^*$  relationships explored in this work is provided in Fig. 5, which illustrates the benefits of using a mixture of CHB + high MW PIB for *n*-octanol sequestration. More generally, it demonstrates the potential of a TPPB polymer formulation based upon carefully selected mixtures of organic solvent and high MW polymer to provide high absorptive capacity while maintaining viscoelastic solid properties.

#### 4. Conclusions

Solute absorption by a polymer is a predictable function of the material's number average molecular weight ( $M_n$ ). However, it is independent of MWD (e.g. unimodal, bimodal). In the context of TPPB polymer selection, thermodynamic affinity for the target solute (PC) encourages the selection of low  $M_n$  material, while mechanical strength ( $G^*$ ) favors high  $M_n$  polymer. Compromises between PC and  $G^*$  inherent to unimodal MWD polymers can be mitigated using bimodal MWD formulations, made from mixing very low MW material (organic solvent or oligomer) with a high MW polymer. The low MW material is selected to maximize solute PC through both entropic and enthalpic contributions, while the

high MW polymer's role is to provide the requisite material strength to the mixture.

#### Acknowledgements

We gratefully acknowledge the financial support of DuPont Canada and the Natural Sciences and Engineering Research Council of Canada. We also wish to thank the Dortmund Data Bank Software & Separation Technology GmbH for their help with NRTL parameter regression.

#### References

- [1] A. Arca-Ramos, G. Eibes, M.T. Moreira, G. Feijoo, J.M. Lema, Vegetable oils as NAPLs in two phase partitioning bioreactors for the degradation of anthracene by laccase, *Chem. Eng. J.* 240 (2014) 281–289, <http://dx.doi.org/10.1016/j.cej.2013.11.076>.
- [2] M. Minier, G. Goma, Ethanol production by extractive fermentation, *Biotechnol. Bioeng.* 24 (1982) 1565–1579, <http://dx.doi.org/10.1002/bit.260240710>.
- [3] H.C. Cheng, F.S. Wang, Computer-aided biocompatible solvent design for an integrated extractive fermentation–separation process, *Chem. Eng. J.* 162 (2010) 809–820, <http://dx.doi.org/10.1016/j.cej.06.018>.
- [4] A.J. Daugulis, D.E. Swaine, F. Kollerup, C.A. Groom, Extractive fermentation-integrated reaction and product recovery, *Biotechnol. Lett.* 9 (1987) 425–430.
- [5] J. Marcoux, E. Déziel, R. Villemur, F. Lépine, J.G. Bisailon, R. Beaudet, Optimization of high-molecular-weight polycyclic aromatic hydrocarbons' degradation in a two-liquid-phase bioreactor, *J. Appl. Microbiol.* 88 (2000) 655–662, <http://dx.doi.org/10.1046/j.1365-2672.2000.01011.x>.
- [6] J. Rocha-Rios, S. Bordel, S. Hernández, S. Revah, Methane degradation in two-phase partition bioreactors, *Chem. Eng. J.* 152 (2009) 289–292, <http://dx.doi.org/10.1016/j.cej.2009.04.028>.
- [7] S.M. Zamir, S. Babatabar, S.A. Shojaosadati, Styrene vapor biodegradation in single- and two-liquid phase biotrickling filters using *Ralstonia eutropha*, *Chem. Eng. J.* 268 (2015) 21–27, <http://dx.doi.org/10.1016/j.cej.2015.01.040>.
- [8] P. Parnian, S.M. Zamir, S.A. Shojaosadati, Styrene vapor mass transfer in a biotrickling filter: effects of silicone oil volume fraction, gas-to-liquid flow ratio, and operating temperature, *Chem. Eng. J.* 284 (2016) 926–933, <http://dx.doi.org/10.1016/j.cej.2015.08.148>.
- [9] W.E. Barton, A.J. Daugulis, Evaluation of solvents for extractive fermentation with *Clostridium acetobutylicum* and the use of poly(propylene glycol) 1200, *Appl. Microbiol. Biotechnol.* 36 (1992) 632–639.
- [10] S. Arriaga, R. Muñoz, S. Hernández, B. Guieysse, S. Revah, Gaseous hexane biodegradation by *Fusarium solani* in two liquid phase packed-bed and stirred-tank bioreactors, *Environ. Sci. Technol.* 40 (2006) 2390–2395, <http://dx.doi.org/10.1021/es051512m>.
- [11] R. Muñoz, S. Arriaga, S. Hernández, B. Guieysse, S. Revah, Enhanced hexane biodegradation in a two phase partitioning bioreactor: overcoming pollutant transport limitations, *Process Biochem.* 41 (2006) 1614–1619, <http://dx.doi.org/10.1016/j.procbio.2006.03.007>.
- [12] J. Harris, A.J. Daugulis, Biocompatibility of low molecular weight polymers for two-phase partitioning bioreactors, *Biotechnol. Bioeng.* 112 (2015) 2450–2458, <http://dx.doi.org/10.1002/bit.25664>.
- [13] T.B. Janikowski, D. Velicogna, M. Punt, A.J. Daugulis, Use of a two-phase partitioning bioreactor for degrading polycyclic aromatic hydrocarbons by a

- Sphingomonas sp., Appl. Microbiol. Biotechnol. 59 (2002) 368–376, <http://dx.doi.org/10.1007/s00253-002-1011-y>.
- [14] A.J. Daugulis, M.C. Tomei, Overcoming substrate inhibition during biological treatment of monoaromatics: recent advances in bioprocess design, Appl. Microbiol. Biotechnol. 90 (2011) 1589–1608, <http://dx.doi.org/10.1007/s00253-011-3229-z>.
- [15] L.D. Collins, A.J. Daugulis, Biodegradation of phenol at high initial concentrations in two-phase partitioning batch and fed-batch bioreactors, Biotechnol. Bioeng. 55 (1997) 155–162, [http://dx.doi.org/10.1002/\(SICI\)1097-0290\(19970705\)55:1<155::AID-BIT16>3.0.CO;2-L](http://dx.doi.org/10.1002/(SICI)1097-0290(19970705)55:1<155::AID-BIT16>3.0.CO;2-L).
- [16] J.L. Rols, J.S. Condoret, C. Fonade, G. Goma, Mechanism of enhanced oxygen transfer in fermentation using emulsified oxygen-vectors, Biotechnol. Bioeng. 35 (1990) 427–435, <http://dx.doi.org/10.1002/bit.260350410>.
- [17] G. Quijano, M. Hernandez, F. Thalasso, R. Muñoz, S. Villaverde, Two-phase partitioning bioreactors in environmental biotechnology, Appl. Microbiol. Biotechnol. 84 (2009) 829–846, <http://dx.doi.org/10.1007/s00253-009-2158-6>.
- [18] B.G. Amsden, J. Bochansz, A.J. Daugulis, Degradation of xenobiotics in a partitioning bioreactor in which the partitioning phase is a polymer, Biotechnol. Bioeng. 84 (2003) 399–405, <http://dx.doi.org/10.1002/bit.10804>.
- [19] G. Quijano, M. Hernandez, S. Villaverde, F. Thalasso, R. Muñoz, A step-forward in the characterization and potential applications of solid and liquid oxygen transfer vectors, Appl. Microbiol. Biotechnol. 85 (2010) 543–551, <http://dx.doi.org/10.1007/s00253-009-2146-x>.
- [20] M.C. Tomei, M.C. Annesini, S. Rita, A.J. Daugulis, Two-phase partitioning bioreactors operating with polymers applied to the removal of substituted phenols, Environ. Sci. Technol. 44 (2010) 7254–7259, <http://dx.doi.org/10.1021/es903806p>.
- [21] S.L. Bacon, J. Scott Parent, A.J. Daugulis, A framework to predict and experimentally evaluate polymer-solute thermodynamic affinity for two-phase partitioning bioreactor (TPPB) applications, J. Chem. Technol. Biotechnol. 89 (2014) 948–956, <http://dx.doi.org/10.1002/jctb.4348>.
- [22] S.L. Bacon, E.C. Peterson, A.J. Daugulis, J.S. Parent, Selecting polymers for two-phase partitioning bioreactors (TPPBs): consideration of thermodynamic affinity, crystallinity and glass transition temperature, Biotechnol. Progr. 31 (2015) 1500–1507, <http://dx.doi.org/10.1002/btpr.2148>.
- [23] J. Rocha-Rios, G. Quijano, F. Thalasso, S. Revah, R. Muñoz, Methane biodegradation in a two-phase partitioning internal loop airlift reactor with gas recirculation, J. Chem. Technol. Biotechnol. 86 (2011) 353–360, <http://dx.doi.org/10.1002/jctb.2523>.
- [24] M. Hernández, G. Quijano, F. Thalasso, A.J. Daugulis, S. Villaverde, R. Muñoz, A comparative study of solid and liquid non-aqueous phases for the biodegradation of hexane in two-phase partitioning bioreactors, Biotechnol. Bioeng. 106 (2010) 731–740, <http://dx.doi.org/10.1002/bit.22748>.
- [25] J.M. Aldric, J.P. Lecomte, P. Thonart, Study on mass transfer of isopropylbenzene and oxygen in a two-phase partitioning bioreactor in the presence of silicone oil, Appl. Biochem. Biotechnol. 153 (2009) 67–79, <http://dx.doi.org/10.1007/s12010-008-8480-0>.
- [26] A. Arwa, S. Baup, N. Gondrexon, J.-P. Magnin, J. Willison, Enhancement of mass transfer characteristics and phenanthrene degradation in a two-phase partitioning bioreactor equipped with internal static mixers, Biotechnol. Bioprocess Eng. 16 (2011) 413–418, <http://dx.doi.org/10.1007/s12257-010-0002-6>.
- [27] H. Fam, A.J. Daugulis, Substrate mass transport in two-phase partitioning bioreactors employing liquid and solid non-aqueous phases, Bioprocess Biosyst. Eng. 35 (2012) 1367–1374, <http://dx.doi.org/10.1007/s00449-012-0725-1>.
- [28] R. Muñoz, M. Chambaud, S. Bordel, S. Villaverde, A systematic selection of the non-aqueous phase in a bacterial two liquid phase bioreactor treating  $\alpha$ -pinene, Appl. Microbiol. Biotechnol. 79 (2008) 33–41, <http://dx.doi.org/10.1007/s00253-008-1400-y>.
- [29] M. Hernández, G. Quijano, R. Muñoz, S. Bordel, Modeling of VOC mass transfer in two-liquid phase stirred tank, biotrickling filter and airlift reactors, Chem. Eng. J. 172 (2011) 961–969, <http://dx.doi.org/10.1016/j.cej.2011.07.008>.
- [30] M.J. Pittman, M.W. Bodley, A.J. Daugulis, Mass transfer considerations in solid-liquid two-phase partitioning bioreactors: a polymer selection guide, J. Chem. Technol. Biotechnol. 90 (2015) 1391–1399, <http://dx.doi.org/10.1002/jctb.4720>.
- [31] P.J. Flory, Principles of Polymer Chemistry, Cornell University Press, New York, 1953.
- [32] B. Guiesse, C.B. Mattiasson, Microbial degradation of phenanthrene and pyrene in a two-liquid phase-partitioning bioreactor, Appl. Microbiol. Biotechnol. 56 (2001) 796–802, <http://dx.doi.org/10.1007/s002530100706>.
- [33] O. Chiantore, Mark–Houwink relationship, in: J. Cazes (Ed.), Encycl. Chromatogr., second ed., Taylor & Francis Group, Boca Raton, FL, 2005, <http://dx.doi.org/10.1081/E-ECHR3-120040043>.
- [34] H. Renon, J.M. Prausnitz, Local compositions in thermodynamic excess functions for liquid mixtures, AIChE J. 14 (1968) 135–144, <http://dx.doi.org/10.1002/aic.690140124>.
- [35] Private Communication with Dortmund Data Bank Software & Separation Technology (DDBST GmbH). <<http://www.ddbst.com>>, 2015.
- [36] T. Lindvig, M.L. Michelsen, G.M. Kontogeorgis, A Flory–Huggins model based on the Hansen solubility parameters, Fluid Phase Equilib. 203 (2002) 247–260, [http://dx.doi.org/10.1016/S0378-3812\(02\)00184-X](http://dx.doi.org/10.1016/S0378-3812(02)00184-X).
- [37] S. Abbott, H. Yamamoto, Computer Program – HSPiP, 2013.
- [38] J. Hildebrand, R.L. Scott, The Solubility of Nonelectrolytes, third ed., Reinhold Publishing Corp, New York, 1950.
- [39] C.M. Hansen, Hansen Solubility Parameters: A User's Handbook, second ed., Taylor & Francis Group, Boca Raton, FL, 2007.
- [40] D. Patterson, Polymer compatibility with and without a solvent, Polym. Eng. Sci. 22 (1982) 64–73.
- [41] J.T. Dafoe, J.S. Parent, A.J. Daugulis, Block copolymers as sequestering phases in two-phase biotransformations: effect of constituent homopolymer properties on solute affinity, J. Chem. Technol. Biotechnol. 89 (2014) 1304–1310, <http://dx.doi.org/10.1002/jctb.4380>.
- [42] G.C. Berry, T.G. Fox, The viscosity of polymers and their concentrated solutions, Adv. Polym. Sci. 5 (1968) 261–357, <http://dx.doi.org/10.1007/BFb0050984>.
- [43] L.J. Fetters, D.J. Lohse, R.H. Colby, Chain dimensions and entanglement spacings, in: J.E. Mark (Ed.), Phys. Prop. Polym. Handb., second ed., Springer, 2006, pp. 445–452, [http://dx.doi.org/10.1007/978-0-387-69002-5\\_25](http://dx.doi.org/10.1007/978-0-387-69002-5_25).
- [44] W. Graessley, The entanglement concept in polymer rheology, Adv. Polym. Sci. 16 (1974) 1–179, <http://dx.doi.org/10.1007/BFb0031036>.
- [45] J.D. Ferry, Viscoelastic Properties of Polymers, third ed., John Wiley & Sons Ltd, New York, 1980.
- [46] US EPA, Estimation Programs Interface Suite for Microsoft Windows. <<http://www.epa.gov/opptintr/exposure/pubs/episuite.html>>, 2015.
- [47] J.L. Ramos, E. Duque, M.-T. Gallegos, P. Godoy, M.I. Ramos-Gonzalez, A. Rojas, et al., Mechanisms of solvent tolerance in gram-negative bacteria, Annu. Rev. Microbiol. 56 (2002) 743–768, <http://dx.doi.org/10.1146/annurev.micro.56.012302.161038>.
- [48] D.C. Kannan, J.L. Duda, R.P. Danner, Application of UNIFAC–vdW–FV model to water–PEO systems, Fluid Phase Equilib. 237 (2005) 86–88, <http://dx.doi.org/10.1016/j.fluid.2005.08.012>.
- [49] A. Jones, D. Kannan, R. Danner, UNIFAC–vdW–FV Computer Program, 2005.