Technical Note

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Polymer–solute interactions in solid–liquid two-phase partitioning bioreactors

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Abstract

BACKGROUND: Biphasic systems with immiscible solvents have been studied for *in situ* product removal, and have shown improvements in bioreactor performance, however, problems associated with solvent biocompatibility, bioavailability and operation have been identified. One alternative is the solid–liquid system in which polymer beads are used, absorbing and removing target compounds from the aqueous phase while maintaining equilibrium conditions. This work aims to identify polymer properties that may be important in polymer selection for selected biotransformation molecules including 2-phenylethanol, cis-1,3-indandiol, iso-butanol, succinic acid and 3-hydroxybutyrolactone.

RESULTS: Relatively hydrophobic compounds (e.g. 2-phenylethanol) tend to be absorbed by polymers better than hydrophilic ones (e.g. iso-butanol) based on partition coefficient tests; values as high as 80 were obtained for the former and <3 for the latter. Owing to the presence of polar functional groups on these compounds, polar polymers such as Hytrel® performed better than non-polar ones such as Kraton®. Crystallinity and intermolecular hydrogen-bonding were also found to be important polymer properties.

CONCLUSION: Polymers showed excellent results in absorbing hydrophobic compounds such as aromatic alcohols, and positive results in absorbing hydrophilic compounds but to a lesser extent. Grafting hydrophilic functional groups onto polymers may be a promising approach for extending polymer uptake capabilities and is currently being investigated.

Keywords: polymer; biotransformation; two-phase partitioning bioreactor; ISPR

INTRODUCTION

Compared with chemical processes, biological processes often suffer from low productivity and low final product concentration, often caused by the toxicity of products and/or substrates. Biphasic systems have been investigated as a possible solution for substrate delivery[1] and *in situ* product removal (ISPR).[2] Although two liquid phase systems have demonstrated improvements in reactor performance, potential drawbacks include solvent bioavailability and toxicity. A promising alternative to overcome these problems is the use of solid polymer beads as an immiscible second phase, taking advantage of polymer biocompatibility, non-bioavailability, and ease of recycle. The use of polymers as a sequestering phase for ISPR purposes was first introduced for the bioproduction of 3-methylcatechol from toluene[3] in which end product inhibition was overcome by the use of polymer beads resulting in enhanced product concentration and productivity.[3] The use of polymers has also shown advantages overcoming multiple inhibitions (both the substrate and product were inhibitory) in the bioconversion of carveol to carvone.[4]

This work aims to better understand the polymer properties that may be important for the uptake/absorption of selected compounds based on an evaluation of the partition coefficient, which is a measure of the solute in the polymer phase relative to the aqueous phase at equilibrium. The compounds selected in this study (Table 1) differ in terms of their molecular complexity, market value, and production scale, and cover a wide range of sectors from commodity goods to high value chemicals.

MATERIALS AND METHODS

Chemicals and Polymers

2-phenylethanol (2-PE), succinic acid and phosphoric acid were purchased from Sigma Aldrich Canada (Oakville, ON, Canada). $MgSO_4$, K_2HPO_4, KH_2PO_4 and $(NH_4)_2SO_4$ were purchased from Fisher Scientific (Oakville, Canada). cis-1,3-indandiol was purchased from TopChem Laboratories (Dublin, Ireland). 3-hydroxybutyrolactone (3-HBL) was purchased from TCI America (Portland, USA). Iso-butanol was obtained from SAFC (St Louis, USA). Polymer properties are listed in Table 2. We thank DuPont Canada, Arkema Canada and Bayer Canada for their kind donations.

Analytics

All concentrations were assayed via HPLC (Varian, Prostar) with either a UV-VIS detector (Varian, Prostar, Model #: PS325) for 2PE (Polaris C18 column, 216 nm), cis-1,3-indandiol (Pursuit XR C-8 column, 220 nm) succinic acid (PL-Hi-Plex column, 220 nm), or a Refractive Index detector (Varian, Prostar, Model #: PS356) for iso-butanol and 3-HBL (PL-Hi-Plex column).

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Table 1. Properties of the selected target compounds

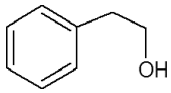
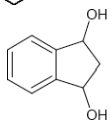
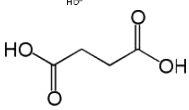
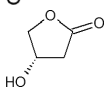
Compound	Industrial application	Structure	CAS number	K_{ow}	Melting point (°C)	Solubility in water
2-phenylethanol	Flavour and fragrance ingredient		60-12-8	37.15	−27	20 g L^{-1} @25 °C
cis-1,3-indandiol	A structural isomer of a pharmaceutical intermediate		172977-38-7	6.31	107	66 g L^{-1} @25 °C
iso-butanol	Potential transportation fuel		78-83-1	5.89	−108	75 g L^{-1} @30 °C
succinic acid	Biorefinery chemical building block		110-15-6	0.18	185	83.2 g L^{-1} @ 25 °C
3-hydroxy-butyrolactone	Biorefinery chemical building block		58081-05-3	0.23	N/A	N/A

Table 2. Properties of polymers used in this study. Beads were approximately spherical with a diameter about 2–5 mm

Trade Name	Grade	Supplier	T_g (°C)	Melting point (°C)	Chemical composition	Specific gravity
Hytrel®	G3548L	DuPont Canada	−45	156	copolymer of poly(butylene terephthalate) and polyether	1.16
	5544		−35	215		1.22
	6108		N/A	168		1.25
	8238		−50	223		1.28
	8206		−59	180		1.19
Zytel®	7304 NC010	DuPont Canada	N/A	220	Polyamide 6	
	42 NC010		70	262	Polyamide 66	1.15
	158 NC010		N/A	217	Polyamide 612	1.06
	RSLC1000 BK385		N/A	203	Polyamide 1010	1.05
PEBAX®	2533	Arkema Canada	−65	134	Polyether block amide	1
	4033		−65	160		1
	7033		N/A	172		1.01
Desmopan®	453	Bayer Canada	−34	145	Thermoplastic polyurethane	1.22
Kraton®	D4150K	Kraton	N/A	N/A	Styrene/butadiene block copolymer	0.92
Elvax®	3175	DuPont Canada	N/A	69	Ethylene/vinyl alcohol	0.95
	770		N/A	96		0.93

Partition coefficient tests

All partition coefficient tests were performed using a typical fermentation medium consisting of (g L^{-1}): $MgSO_4$: 0.5; K_2HPO_4: 1.6; KH_2PO_4: 12.5; $(NH_4)_2SO_4$: 6. The pH of this simulated medium was 5.5. For succinic acid, the pH was adjusted to 3 using phosphoric acid.

2-PE

2-PE concentrations of 1–5 g L^{-1} were prepared by dissolving 2-PE in 10 mL of the simulated medium in 20 mL scintillation vials in triplicate. After polymer addition (approximately 0.46 g) the vials were shaken overnight and the aqueous phase 2-PE concentration was analyzed. As previously done for similar applications,[3,4] the amount of 2-PE absorbed by polymers was calculated by mass balance. The partition coefficient was the slope of the plot of the polymer phase concentration g of 2-PE/L^{-1} of polymer divided by g of 2-PE L^{-1} in aqueous phase, where the volume of polymer is obtained by dividing the mass of the beads by their density. A high degree of linearity in such a plot was obtained, as has been demonstrated previously.[5]

cis-1,3-indandiol

Since cis-1,3-indandiol is more hydrophilic than 2-PE, it was expected that the absorption by polymers would be less than for 2-PE and therefore a single low initial concentration was used. 10 mL of 0.8 g L^{-1} cis-1,3-indandiol were prepared using the simulated medium in 20 mL vials, and polymer beads from 0.5 g to 2 g in 0.5 g increments were then added to each of the vials and the vials equilibrated overnight. Triplicate samples of 0.8 g L^{-1} cis-1,3-indandiol without polymer were prepared as the

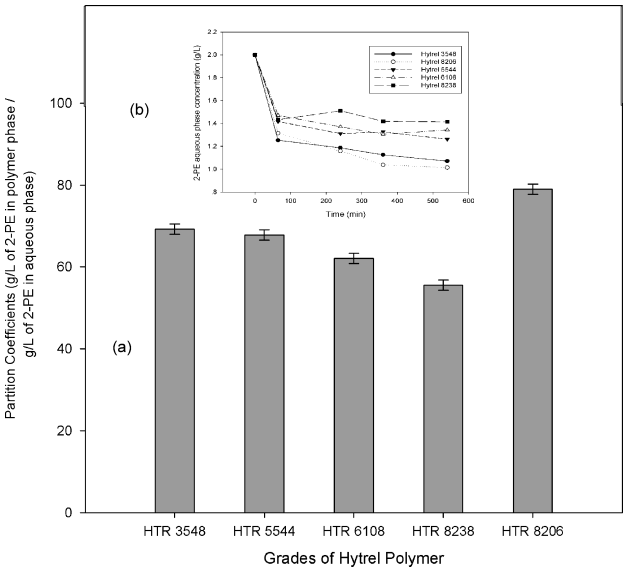


Figure 1. (a) Partition coefficients of different grades of Hytrel® towards 2-phenylethanol, and (b) the decrease of 2-PE concentration in the aqueous phase as a function of time.

blank control and to account for experimental errors. All other procedures were the same as for 2-PE.

iso-butanol

The procedure was similar to that of cis-1,3-indandiol. Initial butanol concentrations of 6 g L^{-1}, 39 g L^{-1}, and 65 g L^{-1} were contacted with polymers added in the range 1 to 3 g L^{-1} in 0.5 g L^{-1} increments.

Succinic acid

The procedure was similar to that of cis-1,3-indandiol. The initial concentration used was 2 g L^{-1}, and the amount of polymer beads added ranged from 1 g L^{-1} to 3 g L^{-1} in 0.5 g L^{-1} increments. The pH of all the samples was adjusted to 3 using phosphoric acid prior to conducting the partitioning tests.

3-hydroxybutyrolactone

The procedure was similar to that of succinic acid. The initial concentration was 10 g L^{-1}, and the amount of polymer beads added ranged from 1 g L^{-1} to 3 g L^{-1} with 0.5 g L^{-1} increments.

RESULTS AND DISCUSSION

2-Phenylethanol

Biologically derived 2-PE is commercially attractive because it can be marketed as a 'natural' product. Produced by *Kluyveromyces marxianus*, it can be toxic as the aqueous phase level approaches 2 g L^{-1}. Solid–liquid biphasic systems are a better option than solvent–aqueous systems for this application because emulsion formation has been reported for the latter[6] and the odour from solvent residues may compromise the product quality. Because 2-PE has a hydroxyl functional group, and because it has been suggested that hydrogen-bonding might be important for polymer uptake[5,7] Hytrel® was selected; the two monomers (polyester and polyether) comprising Hytrel® both have the potential for hydrogen-bonding. The results of the partition coefficient of different grades of Hytrel® are shown in Fig. 1(a) and indicate that all of the Hytrel® grades had high partition coefficients. In fact in a parallel study, a two-phase partitioning bioreactor (TPPB) was used to produce 2-PE using *Kluyveromyces marxianus* and Hytrel® polymer beads as the sequestering phase; compared with other work in the literature in which immiscible organic solvents were used, this study showed significantly better results.[8] The high partition coefficients lead to the hypothesis that one important aspect is the interaction of functional groups between 2-PE and the polymer, in this particular case, through hydrogen-bonding, as also seen for the absorption of phenol[5] and the absorption of 3-methycatechol.[3]

The difference between the various grades of Hytrel® is the proportion of the two monomers, with polybutylene ester increasing in the series Hytrel 3548 < Hytrel 5544 < Hytrel 6108 < Hytrel 8238. The degree of hardness also increases in the same fashion. Except for Hytrel 8206, the partition coefficient decreased as the hardness increased. In Hytrel® copolymer polybutylene ester makes up the crystalline region (the 'hard' segment) while polyether makes up the amorphous region in the polymer chains (the 'soft' segment). By varying the composition of the two, the crystallinity, hence the hardness, of Hytrel® can be altered. The crystallinity of polymers can affect the diffusion of small molecules into polymers[9] and in the amorphous regions of a polymer, both the chain mobility and the free space are larger than in the crystalline regions, and can accommodate molecules that entered the chains better than the crystalline regions. The data obtained from the 2-PE/Hytrel® system showed that polymers with low crystallinity are preferred for small molecule absorption relative to polymers with high crystallinity. Hytrel® 8206 is a special polymer grade designed for polar compound permeation and it did not follow the same trend as the other grades of Hytrel®. The special features of Hytrel® 8206 are unknown for proprietary reasons. Hytrel® 6108 has a modified structure, with the terphthalic acid in the polyester segment being replaced by isophthalic acid (personal communication, DuPont Canada). The incorporation of isophthalic acid disrupted the original regularity in the polyester and reduced the crystallinity of the polyester phase.

Rate tests, either absorbing solute from the aqueous phase or releasing solute into the aqueous phase, are important to the overall bioreactor performance, as shown by several studies.[10,11] In a parallel study the rate of absorption of 2-PE was also measured for each polymer tested, shown in Fig. 1(b). For ISPR fast diffusion into the polymer is preferred because it shortens the time that cells are exposed to a toxic compound. As shown in Fig. 1(b), the two grades of Hytrel® that had the highest partition coefficient towards 2-PE also showed faster uptake, and for all polymer tested, the major reduction of aqueous 2-PE concentration occurred within the initial 100 min. It is expected that under more vigorous agitation in a bioreactor, the absorption could be faster. The successful demonstration of enhanced 2-PE production by ISPR in a solid–liquid TPPB with Hytrel® 8206 has recently been shown.[8]

cis-1,3-indandiol

cis-1,2-indandiol is a chiral intermediate for a commercial drug developed by Merck & Co. for treating HIV[12] and since cis-1,2-indandiol is not commercially available, the close structural isomer cis-1,3-indandiol was used. As seen in Table 1, cis-1,3-indandiol has an aromatic ring and two hydroxyl groups. The rationale for selecting Kraton®, Hytrel® (G3524), Desmopan® and Zytel®. was to test which functional group is more important. From Fig. 2(a) only Hytrel® 3524 showed uptake with a partition coefficient

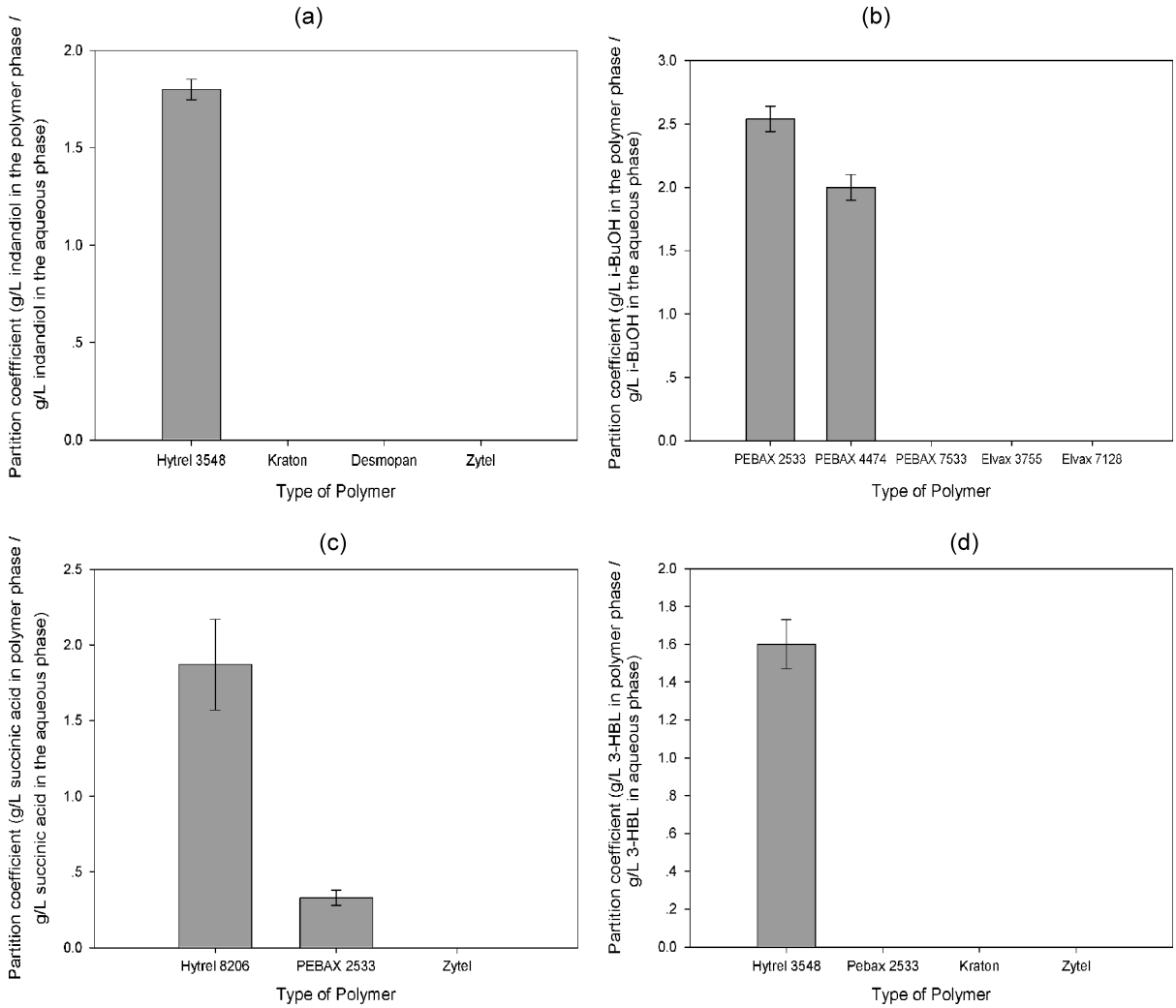


Figure 2. Partition coefficients of candidate polymers towards compounds of interests (a) cis-1,3-indandiol (b)isobutanol (c) succinic acid (d) 3-HBL.

significantly lower than for 2-PE. Hydrophobic polymers with only aromatic functionality such as Kraton® did not work well, suggesting that π-interactions between the aromatic rings of the polymer and cis-1,3-indandiol were not likely mechanisms for absorption. On the other hand, a relatively polar polymer such as Hytrel® showed uptake suggesting that the hydroxyl groups in cis-1,3-indandiol interact with polyether and polyester in the polymer.

iso-butanol

Butanol has a lower vapour pressure, high energy content, and better miscibility with gasoline and diesel than ethanol and can be produced from biomass. Similar to 2-PE, iso-butanol is an alcohol, but is more hydrophilic than 2-PE because of the lack of an aromatic ring. Based on studies using polymer membranes for the pervaporation of butanol,[3,14] polyether block amide (PEBAX®) was found to be among the most effective and at elevated temperature, butanol can dissolve PEBAX® 2533.[3] Thus, three grades of PEBAX® (the difference between the grades of PEBAX® is the proportion of the two monomers) were selected as the candidate polymers together with two grades of Elvax®; it was thought that the acetate group may facilitate hydrogen-bonding with butanol. The uptake results are shown in Fig. 2(b). The proportion of polyamide (the 'hard' segment) increases as PEBAX 2533 < PEBAX 4474 < PEBAX 7533, and the percentage of the other monomer, polyether (the "soft" segment) decreased in the same manner. Therefore, PEBAX® 2533 is the softest and the least crystalline of the three grades. Similar to the 2-PE/Hytrel® system the softer, amorphous grade showed better absorption than the hard, crystalline grade. Elvax® also did not show any uptake, possibly because of the crystalline polyethylene content.

Polymeric resins have also been shown to take up butanol although the uptake mechanism is through surface adsorption[15] instead of whole polymer absorption as is the case in our work. These resins are often brittle and fragile, however, and under high shear conditions in stirred tank vessels, they can easily be broken down.[3] Also, the cost of such resins can be more than 30 times higher than the polymers used here.[15]

Succinic acid

Succinic acid and 3-HBL are potential building blocks for the emerging biorefinery concept. Succinic acid, a dicarboxylic acid with a pKa_1 of 4.2, was expected to be difficult to extract if dissociated, therefore, the tests were performed at a pH that is well below its pKa_1. Different grades of Zytel® (polyamide) were first selected because polyamide is a relatively polar polymer,

however, the results showed no uptake for all the grades tested. Although polyamide has polar amide linkages that can interact with the carboxyl group in succinic acid, the polymer sites may be occupied by intermolecular hydrogen-bonding within the polymer itself. The intermolecular hydrogen-bonding between polymer chains may also make the structure more regular and symmetric, increasing crystallinity. Results for PEBAX® and Hytrel®, shown by Fig. 2(c), indicate that PEBAX® had better results than Zytel® possibly because it is a copolymer of polyamide and polyether. The presence of the 'soft' segment polyether in PEBAX® reduces crystallinity compared with purely polyamide (Zytel®). Hytrel® showed even better results possibly because both monomers in Hytrel® are available for hydrogen-bonding.

3-hydroxybutyrolactone (3-HBL)

As 3-HBL is a fairly polar compound (Table 1) the candidate polymers were Hytrel®, PEBAX®, Zytel® and Kraton®, with results shown in Fig. 2(d). Except for Hytrel® with a partition coefficient of 1.6, the other polymers showed no uptake. Grafting hydrophilic functional groups onto the hydrophobic backbones of polymers can be an option as it has been shown that grafting acrylic acid and methacrylic acid onto an EVOH polymer, increases its permeability towards polar compounds such as methanol. Research in this direction is currently being undertaken in our group.

CONCLUSION

In a copolymer the composition of the 'hard' and 'soft' segment can affect the absorption of small organic compounds, with more 'soft' segments having higher extraction power when other aspects are equal. Hydrogen-bonding between functional groups of small molecules and polymers is important for their interactions and may be the main mechanism for absorption of relatively polar species. Hydrophilic compounds pose challenges for polymer uptake compared to compounds such as 2-PE, however, as relatively polar polymers (e.g. Hytrel®) showed positive results (partition coefficients >1), their extraction is promising. Grafting polar functionality, such as acrylic acid and maleic anhydride, onto the backbone of polymers may be a good option to enhance absorption toward such hydrophilic compounds. ISPR studies for all of the biotransformations discussed here are underway.

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