Solubility of Polyhydroxyalkanoates by Experiment and Thermodynamic Correlations

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This article presents and develops the thermodynamic correlation model to obtain the solubility of polyhydroxyalkanoates (PHAs) in solvents. This model offers a new way to evaluate the polymer solubility parameters and regressions between experimental solubility and solubility parameters. Important parameters influencing solubility such as temperature, polymer molecular weight, crystallinity, and the kind of PHAs were studied. Experiments were carried out on PHB-co-Hx copolymers of different contents. Finally, a model which takes into account these parameters were established and tested on 232 experimental points. The method developed could be used for the selection of a solvent with high efficiency, low price, and low toxicity. © 2007 American Institute of Chemical Engineers *AIChE J,* 53: 2704–2714, 2007
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Introduction

Nowadays, the plastic materials are taking an important place in product design and engineering. Their varied physical properties make them very convenient in applications. However, these nondegradable plastics are accumulating in the environment at the rate of 25 million tones per year.[1] That is why the replacement solutions like the using of biopolymers (biodegradable polymers) have been envisaged. On that purpose, different kinds of polyesters—polyhydroxyalkanoates (PHAs), polylactides (PLAs), aliphatic polyesters, and polysaccharides have been studied for the last 80 years. PHAs have some very interesting physical properties and biodegradability. In fact, they could be used in packaging as well as in medical applications because of their biocompatibility and slow hydrolytic degradation.[2] The structure of PHAs is shown in Figure 1. Since the discovery by Lemoigne, more than 80 different monomer units have been detected in various bacteria,[3] HV, HB, and HX are the more common ones. Three main techniques have been studied to produce these biopolymers. These are chemical synthesis, using transgenic plants cells and bacterial fermentation.

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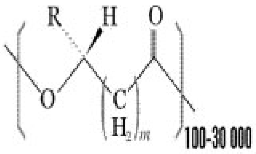

n = 1	R = hydrogen	poly(3-hydroxypropionate)	P(3HP)
	R = methyl	poly(3-hydroxybutyrate)	P(3HB)
	R = ethyl	poly(3-hydroxyvalerate)	P(3HV)
	R = propyl	poly(3-hydroxycaproate)	P(3HC)
	R = butyl	poly(3-hydroxyheptanoate)	P(3HH)
	R = pentyl	poly(3-hydroxyoctanoate)	P(3HO)
	R = hexyl	poly(3-hydroxynonanoate)	P(3HN)
	R = heptyl	poly(3-hydroxydecanoate)	P(3HD)
	R = octyl	poly(3-hydroxyundecanoate)	P(3HUD)
	R = nonyl	poly(3-hydroxydodecanoate)	P(3HDD)
n = 2	R = hydrogen	poly(4-hydroxybutyrate)	P(4HB)
n = 3	R = hydrogen	poly(5-hydroxyvalerate)	P(5HV)

Figure 1. Structure of PHAs.[3]

Through the bacterial fermentation approach, PHA is produced in the cytoplasm of the cell. Some bacteria, such as *Escherichia coli*, under limitation of nitrogen, phosphorus, magnesium, or oxygen, metabolizes carbon source substrates into PHA-granules. In 1962, a French microbiologist Lemoigne[4] discovered the first variety of PHA in *Bacillus megaterium*: the polyhydroxybuyrate P(3HB). Many comparisons have been made between P(3HB) and conventional petrochemical plastics, like isotactic polypropylene.[2] The results show that P(3HB) has useful properties such as moisture resistance, piezoelectricity, and a specific optical density.[3] But, this polymer also has two major drawbacks: its brittleness after several days at ambient conditions and its poor melting stability (the degradation temperature and the melting point are very close). To solve these problems, blends of PHAs and PHAs copolymers were introduced (Table 1). At the end of 1980, Imperial Chemical Industries (ICI) first sold this new kind of copolymer (poly(3-hydroxybutyrate-*co*-hydroxyvalerate, (PHB-*co*-HV)) under the commercial name BIOPOL™.

One of the most important problems of PHAs was their high price, in comparison with petrochemical plastics, such as polyethylene and polypropylene.[1] Much research has been done to make the PHAs' industrial production more competitive. On that purpose, more attention has to be put on purification of these biopolymers, which could represent from 60 to 80% of the total cost. Solvent extraction is one of the popular methods used to achieve high purity levels in PHAs isolation and purification. One of the problems of the use of solvent comes from their toxicity and price, especially when large amount of solvent is needed. The purpose of this article is to develop a model to predict the solubility of PHB and PHB copolymers in various solvents, in order to select a reasonably priced, green, and efficient solvent. This method could also be used to find appropriate solvents for the elimination of endotoxin which is present in some Gram-negative bacteria. The presence of this toxin in PHA is a potential problem for its use in medical applications.[6]

Experimental Section

Materials

Most of the solvents used were of analytical grade or have purity higher than 98%. The solvents were chosen to study the solubility behavior of different organic functions on PHAs. Several samples from P(3HB) were used. Table 2 gives the properties of these polymers. The low molecular weight polymer (M_w = 4500) was obtained by chemical digestion of the sample from Jiangsu Nantian (M_w = 80,000, P.R. China). The digestion was conducted in the presence of sodium hypochlorite (7.3 wt %) at 40°C during 2 h. Then the reaction was quenched with cold hexane, and PHB was recovered by Buchner filtration.

Solubility measurements

For each sample, 10 cm^3 of the studied solvent were placed into a 50 cm^3 test tube and 0.1 g of P(3HB) was added. The mixture was then shacked at 120 rpm in a thermostated bath for 1 h at 70°C, to activate the solubilization of the polymer, and then 2 h at the selected temperature (from 30 to 70°C). The test tubes were put into a thermostated bath for at least 12 h to make a decantation by gravity. For some samples, such as 1,2-dichloroethane, the decantation was not easy to accomplish, that is why a filtration step is added, in order to eliminate the polymer in suspension. The sealing of the test tube was provided by the use of a PTFE film (Teflon™). Finally, 3 cm^3 of the upper liquid part of the mixture were put on a preweighted Petri dish (ϕ, 60 mm). The solvent was then evaporated into a fume hood until the sample is totally dry. Then, polymer residues were

Table 1. Comparison of Polymers Properties[3,5]

Polymer	Melting Temp., °C	Young's Modulus, GPa	Tensile Strength, MPa	Elongation to Break, %
P(3HB)	179	3.5	40	5
P(3HB-*co*-3HV)				
3 mol % 3HV	170	2.9	38	–
9 mol % 3HV	162	1.9	37	–
14 mol % 3HV	150	1.5	35	–
20 mol % 3HV	145	1.2	32	50
25 mol % 3HV	137	0.7	30	–
Polypropylene	170	1.7	34.5	400
Polyethyleneterephtalate	262	2.2	56	7300
Polystyrene	110	3.1	50	–

Table 2. PHB Properties

Company	Purity, %	M_w	T_m, °C	Crystallinity
Aldrich Standart	100.0	670,000	175	0.58
Nantian (China)	99.2	1,300,000	176	0.48
Nantian (China)	97.2	690,000	171	0.53
Nantian (China)	98.5	250,000	172	0.83
Nantian (China)	98.2	80,000	166	0.35
Nantian (China)*	–	4500	155	0.49

*Degraded with sodium hypochlorite from Nantian M_w = 80,000 P(3HB).

weighed with a precision of 10^{-4} g. In doing these measurements, the decanted samples must be saturated, that is why an higher concentrations may be used for good solvents like chloroform ([P(3HB)]= 50 g/L) or for the case of copolymers. For each experiment, the samples and solubility measurements were duplicated, the data collected had standard deviation lower then 10%.

Molecular Weight Determination. The molecular weights of different polymers used were measured using gel permeation chromatograph. Two columns of different sizes were used for the analysis: 10,000–600,000 and 200,000–2,000,000. All polymers were diluted in chloroform with an average concentration of 0.1 wt %. Elution was done in hot chloroform (40°C) at the flow rate of 1 cm^3/min. The calibration of the apparatus was performed with five polystyrene standards

Determination of Crystallinity. The crystallinity was tested by thermal analysis with differential scanning calorimetry (DSC) (Perkin Elmer DSC7), under an atmosphere of nitrogen. The polymer sample was first heated from −40°C to 200°C (10°C/min), the temperature is then held for 5 min before cooling to −40°C (10°C/min). Temperature is also held for 5 min before a second heating in the same conditions as the first step. The crystallinity (X_c) of PHAs is calculated according to the Eq. 20:[7]

$$X_c = \frac{\Delta H_m}{\Delta H^0{}_{\mathrm{PHB}}} \tag{1}$$

where ΔH_m is the melting enthalpy of the sample (J/g), ΔH_{PHB} is the melting enthalpy of the 100% crystalline PHB which is assumed to be 146 J/g.[7–9] The apparatus was calibrated with Indium (m.p.= 156.61°C and ΔH=28.54 J/g).

Theoretical of Solubility Predictions

Solubility predictions were first described by Hildebrand in 1936, he introduced a solubility parameter (δ_t), which is derived from the cohesive energy density.[10] Improvements of the Hildebrand parameter are based on this idea. The evolution of solubility parameters through the years is shown in Figure 2. In 1967, Hansen proposed a further extension of Prausnitz and Blanks 2-parameter model by separating the single polar contribution into two, one due to a distinct polar factor (δ_p), and one due to hydrogen bonding (δ_h) contributions.[11,12] And the third parameter (δ_d) is called the dispersion component. The three-component parameter proposed by Hansen has been the most widely used for the empirical description of polymer–liquid systems. In addition, there are several other variations developed for practical applications, but the method that provides an acceptable compromise between rigor and simplicity is the one developed by Beerbower et al.[13] To get accurate results, the correlation must be based on solubility parameters adaptable to different temperatures. In 2004, Williams et al.[14] showed that the solubility parameters could be recalculated for different temperatures and pressures.

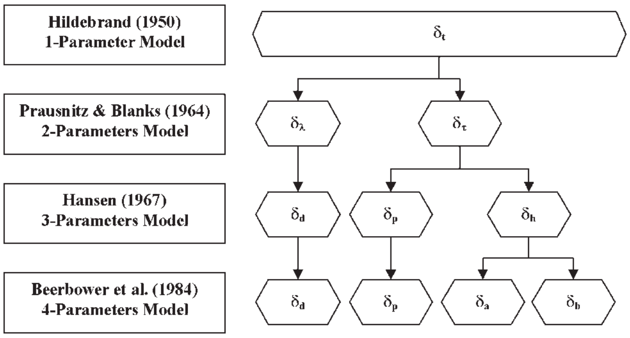

Figure 2. Principal evolutions of empirical solubility parameter models.[11]

Evaluation of PHB solubility parameters

Group Contribution Methods. The Hansen parameters could be determined by the Krevelen group contribution method. Each parameter could be linked to the group molar attraction constant as shown in Table 3 by the following equations:[15,16]

$$\delta_d = \frac{\sum F_d}{V_m} \tag{2}$$

$$\delta_p = \sqrt{\frac{(\sum F_p^2)}{V_m}} \tag{3}$$

$$\delta_h = \sqrt{\frac{\sum E_h}{V_m}} \tag{4}$$

It is obvious that the accuracy of this method is not very high. Solubility parameters obtained with two different calculation methods, Kervelen and Hoy, (Table 4) lead to quite different values

Experimental Methods. For the last 50 years, different methods for evaluating experimental parameters of polymers have been reported. Measurable properties, such as intrinsic viscosity,[17] turbidimetry,[18] weight gain,[19] or swelling[20] of a polymer film, were reported. Verheyen et al.[21] also reported

Table 3. Group Molar Attraction Constants[15,16]

Group	F_d, ($J^{1/2}$ $cm^{3/2}$ mol^{-1})	$F_p{}^2$, ($J^{1/2}$ $cm^{3/2}$ mol^{-1})	E_h, (J/mol)
—COO—	390	240,100	7000
CH_3—	420	0	0
—CH_2—	270	0	0
—CH<	84	0	0

Table 4. PHB Solubility Parameters Obtained With Different Methods[15]

Polymer, P(3HB)	d_d, (MPa$^{1/2}$)	d_p, (MPa$^{1/2}$)	d_h, (MPa$^{1/2}$)
Group contribution methods			
Kervelen*	15.9	6.7	9.8
Hoy*	15.2	11.5	7.6
Experimental methods			
Busamante formula[†,‡]	18.7	6.0	5.1
Barycentric method[†]	19.3	5.3	6.3

*Data from Terada and Marchessault at 25°C.[15]
[†]Value obtained for M_w = 690,000 at 50°C.
[‡]Evaluated with a selection of eight solvent which cover the solubility range.

a correlation between the experimental solubility and solubility parameters. The following correlation was given by Verheyen et al.:[21]

$$\frac{\ln \alpha_2}{U} = C_0 + C_1\delta_{1d}^2 + C_2\delta_{1d} + C_3\delta_{1p}^2 + C_4\delta_{1p} + C_5\delta_{1a} + C_6\delta_{1b} + C_7\delta_{1a}\delta_{1b} \quad (5)$$

where the subscripts 1 and 2 refer to the solvent and the solute, respectively. The solute parameters can be calculated from the resulting coefficients of the regression in Eq. 5.

$$\delta_{2d} = -\frac{C_2}{2C_1} \quad \delta_{2p} = -\frac{C_4}{2C_3} \quad \delta_{2a} = -\frac{C_6}{C_7} \quad \delta_{2b} = -\frac{C_5}{C_7} \quad (6)$$

The activity coefficient is defined as the ratio of the ideal mole fraction solubility (X_2^{id}) to the experimental mole fraction solubility (X_2) of the solute in the solvent.

$$\ln\ \alpha_2 = \ln\left(\frac{X_2^{id}}{X_2}\right) \quad (7)$$

Busamante et al.[22] in 1993 show that solubility parameters can also be correlated to ln X_2, the following model was reported:

$$\ln\ X_2 = C_0 + C_1\delta_{1d}^2 + C_2\delta_{1d} + C_3\delta_{1p}^2 + C_4\delta_{1p} + C_5\delta_{1a} + C_6\delta_{1b} + C_7\delta_{1a}\delta_{1b} \quad (8)$$

This method is not very easy to use, to get a statistical answer—which takes into account the results obtained with all the 20 solvent studied—a strong matrix calculation is involved. To solve this problem, a new method to evaluate the polymer solubility parameters was tested in this article.

In this study, the evaluation of PHA solubility parameters will be based on a barycentric method. Each solvent (Δ_j) is defined by its solubility parameter ($^j\delta_i$) (δ_i = δ_d, δ_p, δ_h, δ_a, δ_b, or δ_t), and the respective "mass coefficient" will be attributed according to the experimental solubility (s_j = s_1, s_2, s_3, . . . , s_n) of each polymer–solvent system. The polymer (Π) position will be defined as the barycenter of the solvent map in Figure 3. Table 5 gives the solubility parameters and the molar volume of the 20 solvents which were studied. Table 4 lists that the values obtained by experimental methods are quite comparable. These parameters are a little different from the group contributions methods. A part of this difference could be explained by the fact that the experimental values have been evaluated at 50°C, whereas Hoy and Kervelen values are given at 25°C.

Results and Discussion

Comments on solubility results

Experimental results showed that some halogenated solvents such as chloroform and dichloromethane have high solubilizing properties. These observations confirm the results obtained by Vanlautem and Gilain.[23] They investigated PHA extractions from *Ralstonia eutropha*, and found that the best results were obtained for solvents in which carbon atoms carrying at least one chlorine atom and one hydrogen atom.[23] This behavior could be explained by a polar interaction between the chloride atom and the carbon that holds the carbonyl function. Moreover, the hydrogen atom of the halogenated compound, which has a lack of electrons, is linked by the carbonyl function of the polymer. Figure 4 shows this interplay of interactions. Moreover, this representation is suggested to be stable according to its five-membered ring structure. This property, which is usually observed when hydrogen is linked to oxygen or nitrogen could also happen when the hydrogen is held by a carbon which is bound to several electronegative atoms. This interpretation is confirmed by Terada and Marchessault,[15] who noticed that tetrachloromethane (CCl_4), an halogenated compound which did not hold an hydrogen atom, was a bad P(3HB) solvent. Reversely, the lower solubility of 1,2-dichloroethane could be explained by the presence of only one chloride on the carbon that hold the hydrogen, and the electronegativity of this carbon is not enough to lead to the creation of an hydrogen bonding. The low solubility of ester and acetone is also explained by their low hydrogen bonding abilities.[10] On the other hand, in the case of alcohols and carboxylic acids this hydrogen bonding is too strong to lead to high solubilizing abilities. For PHB, its solution parameter is δ_h = 6.3. Whereas, for methanol, ethanol, 1-butanol, and acetic acid, the respective values are 20.9, 18.1, 14.9, and 12.7 (at 50°C).

Solubility and solubility parameters

In this study, solubility was predicted by using the solubility radius of the given polymer–solvent system. According to the fact that components with similar solubility parameters have high solubilizing abilities, the solubility radius gives a

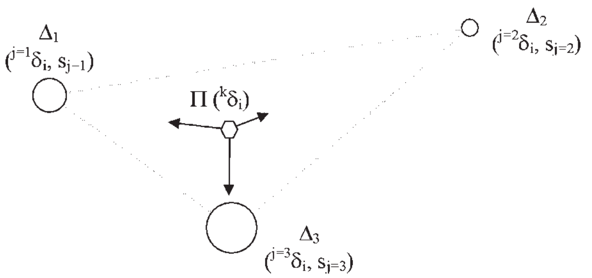

Figure 3. Solvent map in the case of three solvents (Δ_1, Δ_2, Δ_3) and one polymer (Π).

Table 5. Hansen and Beerbower's Parameters for Liquids at 25°C[12]

Solvent	δ_d, MPa$^{1/2}$	δ_p, MPa$^{1/2}$	δ_a, MPa$^{1/2}$	δ_b, MPa$^{1/2}$	δ_h, MPa$^{1/2}$	δ_t, MPa$^{1/2}$	V_m, cm^3/mol	Solvent Number
Hydrocarbons								
n-Hexane	14.9	0.0	0.0	0.0	0.0	14.9	131.6	1
n-Octane	15.3	0.0	0.0	0.0	0.0	15.3	147.4	2
Cyclohexane	16.8	0.0	0.0	0.0	0.0	16.8	108.7	3
Aromatic compounds								
Benzene	18.4	1.0	1.4	1.4	2.0	18.5	89.4	4
Toluene	18.0	1.4	1.6	1.2	2.0	18.2	106.8	5
Halogenated compounds								
1,2-Dichloroethane	19.0	7.4	4.1	2.0	4.1	20.8	79.4	7
Chloroform	17.8	3.1	6.1	2.7	5.7	18.9	81.0	15
Dichloromethane	18.2	6.3	6.4*	2.9*	6.1	20.2	102.0	19
Ethers								
Diethyl ether	14.5	2.9	1.0	12.9	5.1	15.6	105.0	6
Esters								
Ethylacetate	15.1	5.3	10.8	3.9	9.2	18.5	98.5	10
n-Butylacetate	15.8	3.9	6.3	5.7	3.5	16.6	131.5	12
Ketones								
Acetone	15.5	10.4	4.9	4.9	7.0	19.9	74.0	8
Alcohols								
Methanol	15.1	12.3	17.2	22.3	22.3	29.6	41.0	9
Ethanol	15.8	8.8	17.0	11.3	19.4	26.5	58.7	17
1-Butanol	16.0	5.7	13.1	9.4	15.8	23.2	92.8	16
Acids								
Acetic acid	14.5	8.0	14.3	6.3	13.5	21.4	58.0	11
Nitrogen compounds								
N,N dimethylformamide	17.4	13.7	7.0	9.0	11.3	24.9	77.4	13
Aniline	19.4	5.1	3.9	13.3	10.2	22.5	91.5	14
Pyridine	19.0	8.8	2.9	6.6	6.1	21.8	80.9	18
Water	15.6	16.0	13.7	65.5	42.3	47.8	18.0	20

*Evaluated by comparison with chloroform.

good indication of the behavior of a given mixture. Solubility radius of a polymer(*k*)–solvent(*j*) system can be calculated as follows:[24]

Hildebrand radius (1-parameter model):

$$ {}^{kj}r = |{}^{k}\delta_t - {}^{j}\delta_t| \tag{9} $$

Hansen radius (3-parameters model):

$$ {}^{kj}r = [4({}^{k}\delta_d - {}^{j}\delta_d)^2 + ({}^{k}\delta_p - {}^{j}\delta_p)^2 + ({}^{k}\delta_h - {}^{j}\delta_h)^2]^{1/2} \tag{10} $$

Beerbower radius (4-parameters model):

$$ {}^{kj}r = [({}^{k}\delta_d - {}^{j}\delta_d)^2 + ({}^{k}\delta_p - {}^{j}\delta_p)^2 + 2|({}^{k}\delta_a - {}^{j}\delta_a)({}^{k}\delta_b - {}^{j}\delta_b)|]^{1/2} \tag{11} $$

By plotting experimental solubility versus the solubility radius, an exponential decay relation was observed and a limiting radius could be evaluated. (This notion of limiting radius could be compared with the radius of the spherical solubility window proposed by Hansen[15,24]). In this study these curves was regressed with a software program (Sigma plot 8.0) by using a single three parameters exponential decay model (*s*, experimental solubility and *r*, solubility radius) as the following:

$$ s = s_0 + A\ \exp(-Br) \tag{12} $$

To avoid negative values of solubility, the constant s_0 has been assumed to be equal to 0. Figure 5 shows such correlation in Eq. 12 with the three different models in Eqs. 9–11, these results were obtained for a polymer (P(3HB)) molecular weight of 690,000 and a temperature of 50°C.

Discussion on the model

The comparison of the experimental solubilities against the calculated solubility radii gives an idea of the accuracy of the prediction that could be obtained by the different models mentioned earlier. Examining the correlations, it is obvious that the regressions obtained by the 4- and the 3-parameter models bring more accuracy than by the 1-parameter model.

Then, with the radius calculated using Hansen parameters, the corresponding correlation coefficients are between 0.78

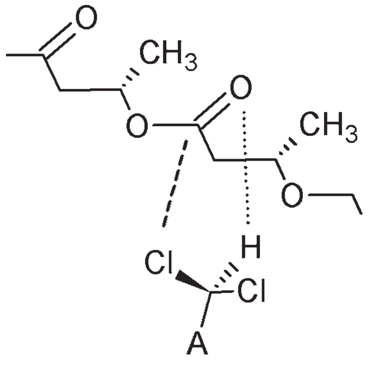

Figure 4. Interactions between P(3HB) and some halogenated compounds.

Chloroform: A = Cl, Dichloromethane: A = H.

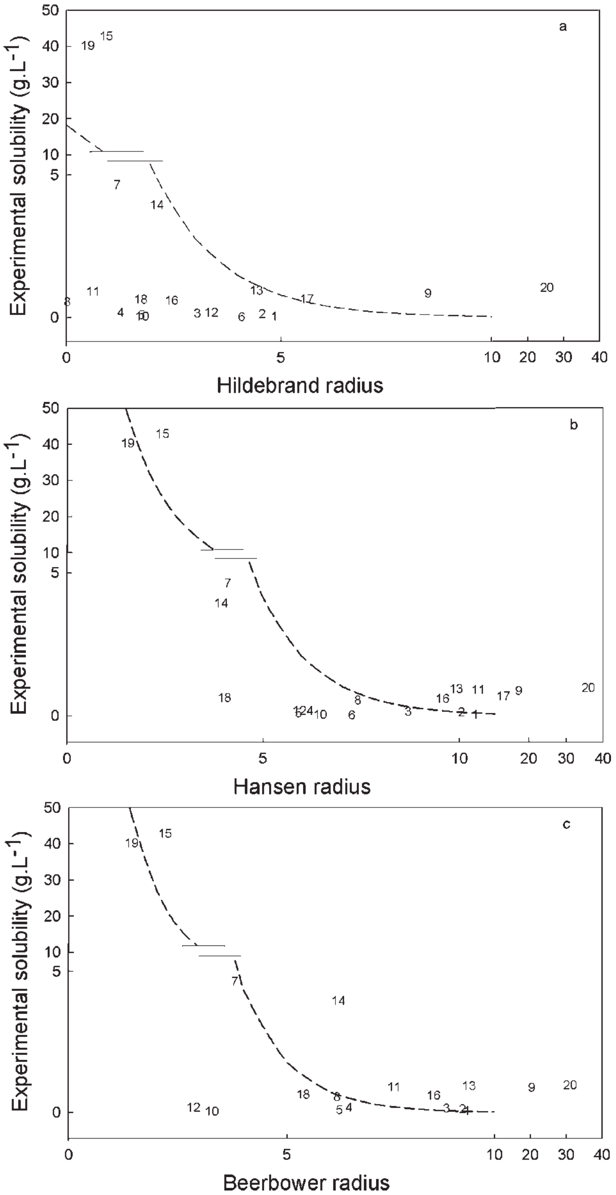

Figure 5. Representation of the experimental solubility of PHB (50°C, M_w 690,000) by using different models.

Each number refers to the solubility of a PHB-solvent system. The regression curves were established according to Eq. 12.

and 0.95 whereas, that for the Beerbower parameters (Table 6 and Figure 5) are between 0.49 and 0.81 (Table 6).

Predictions made by using Beerbower and Hansen models could also be compared with the respective position of some bad and good solvents on Figures 5b, c, and Table 7.

In the case of esters (ethylacetate and *n*-butylacetate, solvent number 10 and 12, respectively), a better prediction is obtained with the Hansen model. For these two bad solvents, the radius calculated with the Hansen parameters are 6.5 and 5.9, respectively, whereas with Beerbower parameters much lower values (3.3 and 2.9) were obtained. The same conclusion could be obtained by comparing the position of aniline (solvent number 14) in these two models. In fact, the prediction of Hansen model provides a smaller radius for this good solvent. On the other hand, the Beerbower model brings more accuracy in the prediction of pyridine (solvent number 18) with a larger radius.

In 1999, Terada and Marchessault[15] reported that acetone and ethylacetate, according to their predictions, should be two good solvent for PHB, judging by the fact that they are located inside the spherical solubility window of Hansen diagram. The radius obtained for these two solvents were 2.1 and 3.8, respectively. In this study, the radii of acetone and ethylacetate (solvent number 8 and 12) are evaluated to 7.4 and 6.5, respectively, with Hansen model. This better prediction could be in part explained by the higher accuracy in the evaluation of PHB solubility parameters. Contrary to this study, which is based on experimental PHB solubility parameters, Terada and Marchessault[15] used the average value of Hoy and Kervelen group contribution methods. (A comparison of these methods is given in Table 4.)

Effect of molecular weight

It is reasonable to assume that polymer solubility parameters are varying with the molecular weight of the polymer. The solubility parameters in various solvents of P(3HB) with different molecular weights were reevaluated. Experiments show that the solubility is large for low-molecular weight polymer. The largest solubility variation is observed for molecular weight lower then 250,000. In the case of PHB-good solvents, a lower viscosity is observed for low-molecular weight polymer solutions at a comparable concentration.

Sadowski[25] also reported that polymer solubility is a strong function of polymer molecular weight and that small polymer chains are more soluble and need a smaller pressure to dissolve than long polymer chains. Moreover, further stud-

Table 6. Parameters of Eq. 12 at Different Molecular Weights and Temperatures

Effect of molecular weight at T = 50°C					
M_w	4500	80,000	250,000	690,000	1,300,000
Hildebrand model					
R^2	0.330	0.401	0.661	0.191	0.555
A_M	72.8	58.9	128.9	18.4	56.4
B_M	1.001	1.483	3.466	0.630	2.574
Hansen model					
R^2	0.845	0.787	0.904	0.852	0.955
A_M	449.2	213.5	178.8	132.4	169.5
B_M	0.680	0.629	0.727	0.694	0.826
Beerbower model					
R^2	0.493	0.536	0.700	0.780	0.812
A_M	414.7	199.8	401.4	164.4	570.2
B_M	0.723	0.724	1.370	0.907	1.696
Effect of temperature at M_w = 690,000					
T, °C	30	40	50	60	70
Hildebrand model					
R^2	0.224	0.215	0.191	0.239	0.213
A_T	19.8	18.9	18.4	24.6	24.0
B_T	0.637	0.592	0.630	0.668	0.576
Hansen model					
R^2	0.835	0.814	0.852	0.819	0.805
A_T	118.9	115.7	132.4	131.4	154.1
B_T	0.675	0.661	0.694	0.657	0.680
Beerbower model					
R^2	0.687	0.669	0.780	0.706	0.696
A_T	120.1	97.6	164.4	103.0	125.2
B_T	0.828	0.725	0.907	0.677	0.708

Table 7. Values of the Solubility Radius, of Various Solvents for PHB

Method	Ethylacetate	*n*-Butylacetate	Aniline	Pyridine	Acetone
Hansen radius*	6.5	5.9	3.9	4.0	7.4
Beerbower radius*	3.3	2.9	6.2	5.4	6.1
Hansen radius calculated by Terada and Marchessault[15]	3.8	–	9.0	7.3–0.6	2.1

*Calculated using Eqs. 10 and 11 at 50°C and $M_w = 690{,}000$.

ies have been done by Behme et al.,[26] considering the molecular weight distribution in the case of polydisperse polymers.

Figure 6 shows experimental points and the regressed curves of the solubility radius against the experimental solubility for different molecular weight PHB samples. Theses curves were established according to Eq. 12. The increasing of solubility when the polymer chains are becoming longer could also be observed; short polymer chains lead to higher solubility curves and long polymer chains lead to low solubility curves. It is also important to notice that the solubility curves are quite similar for high-molecular weight polymers, i.e., very close to each other. This behavior is also shown by the following regression of the constant A_M from Eq. 12, following the same single 3 parameters exponential decay model ($R^2 = 0.979$) (Figure 7).

$$A_M = A_{M0} + C_M \exp(-D_M M_W) \tag{13}$$

A_M, B_M, C_M, D_M, and A_{M0} are the constants obtained by regressing the molecular weight data. Parameter B_M is assumed to be independent of molecular weight (M_w) and equal to 0.711. Then, the experimental solubility could be expressed for a given molecular weight PHB with this formula:

$$s = (A_{M0} + C_M \exp(-D_M M_W)) \exp(-B_{Mr}) \tag{14}$$

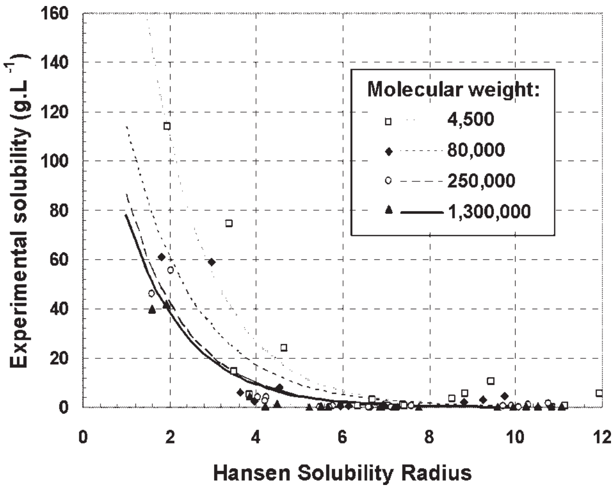

Figure 6. Evolution of the regression curve (24) with the polymer molecular weight (operating temperature 50°C).

Each point (□, ◆, ○, and ▲) represents the experimental solubility of a given polymer–solvent system.

$$s = (159.2 + 292.7 \exp(-0.210 \times 10^{-4} \times M_W)) \exp(-0.711r) \tag{15}$$

This formula is composed of two parts: one (A_M) is dependent from the molecular weight and this other part is only dependent from the solubility radius.

Effect of temperature

In this part, the impact of another important parameter, temperature, was studied.

In general, the solubility measurements were done at the atmospheric pressure, but during the preparation of the sample and the solubilization of the polymer, an increase of the pressure occurs due to the sealing of the test tube. When the test tube was opened, both temperature and the solubility decreased (Figure 8). That is why the solubility reaches a maximal value when the ebullition temperature of the solvent is attained. As this problem is not observed for most of the solvents, we will consider that our solubility is a little lower than the real value. It is also important to note that the solubility parameters of the polymer are following the same evolution as solvent parameters when the operating temperature is varying.

By using the correlation described in Eq. 12, we can get the values of A_T and B_T from Table 6. Figure 9 shows the evolutions of these parameters in a temperature range from $T = 30$ to 70°C. In Figure 9, we notice that A_T coefficient is rising when temperature increases. This behavior could be regressed by using a three parameters exponential rising model by following a similar method as in the molecular weight study. A_T, B_T, C_T, $D_{T,}$ and A_{T0} refer to regressed coefficient obtained in the temperature study.

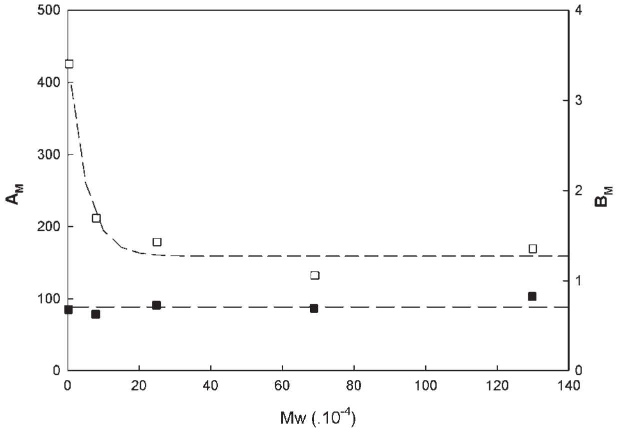

Figure 7. Variation of the regression coefficients A_M (□) and B_M (■) with the polymer molecular weight (operating temperature 50°C).

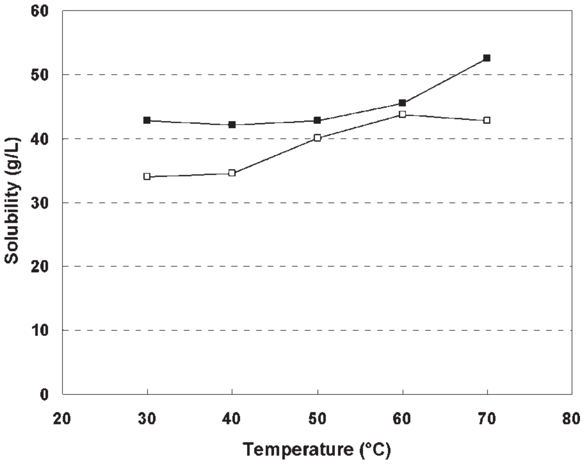

Figure 8. Variation of solubility with temperature for chloroform (■) and dichloromethane (□) (PHB M_w 690,000).

$$s = (A_{T0} + C_T \exp(D_T T)) \exp(-B_{Tr}) \tag{16}$$

$$s = (114.8 + 0.454 \exp(0.063T)) \exp(-0.673r) \tag{17}$$

It is important to note that the value of the coefficient B_T in the temperature study ($B_T = 0.673$) could be considered as a constant which is very similar to value of the coefficient B_M calculated in the molecular weight study ($B_M = 0.711$). So, by using an average value of the coefficient B, we could express the solubility with a term independent of temperature (°C) and of the polymer molecular weight (M_w).

$$s = f(M_W, T) \exp(-0.692r) \tag{18}$$

Then, based on the results obtained from a common experiment of the two studies (50°C and M_w = 690,000), the term function of temperature and of the molecular weight could be expressed as follows:

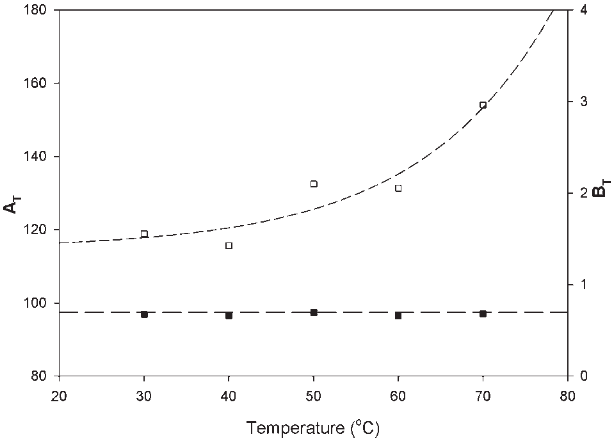

Figure 9. Variation of the regression coefficients A_T (□) and B_T (■) with temperature (M_w = 690,000).

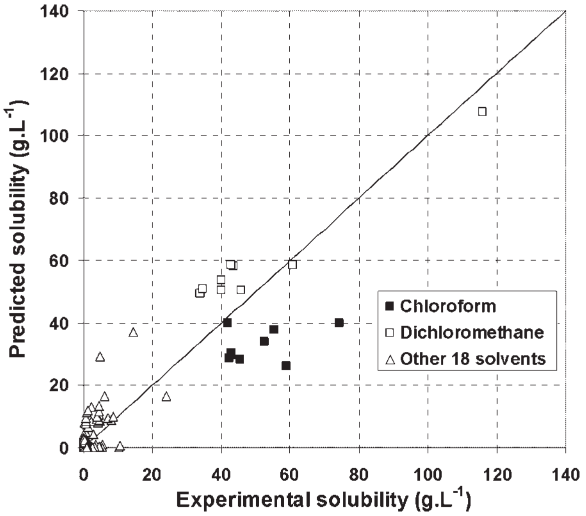

Figure 10. Predicted and experimental solubility values of 175 solvent–polymers systems with polymer molecular weight from 4500 to 1,300,000 and temperature between 30 and 70°C.

$$s = (141.7 + 0.454 \exp(0.063T) + 292.8 \exp(-0.210\times10^{-4} \times M_W)) \exp(-0.692r) \tag{19}$$

This formula was established by assuming that the solubility behavior obtained between 30 and 70°C with a molecular weight of 690,000 could be applied to all other molecular weights. In our working condition, this formula is only applicable to temperature lower then the ebullition temperature of the solvent. Figure 10 compares the results predicted from the formula with the 175 experimental points obtained with polymer–solvent systems (temperature range from 30 to 70°C and molecular weight range from 4500 to 1,300,000).

Polymer crystallinity

After examination of dry bacterial PHB by transmission and scanning electron microscopy, Lauzier et al.[27] showed that granules consist of two different components: a solid shell composed of overlapping lamellar crystals and a soft noncrystalline core. This morphology comes from the fact that in vivo PHA molecules are in an amorphous rubbery state. These PHA granules are covered by a 4-nm-thick surface layer composed of proteins and phospholipids.[28–32] The

Table 8. Properties of PHB-*co*-Hx Copolymers Used in This Study

Company	Comonomer, %	M_w	T_m, °C	Crystallinity
P(3HB-*co*-3HX)				
Nantian (China)	0	690,000	171	0.53
Procter & Gamble (US)	3.9	967,000	139/153	0.43
Procter & Gamble (US)	7.3	771,000	131/142	0.50
Procter & Gamble (US)	10.5	427,000	128	0.31

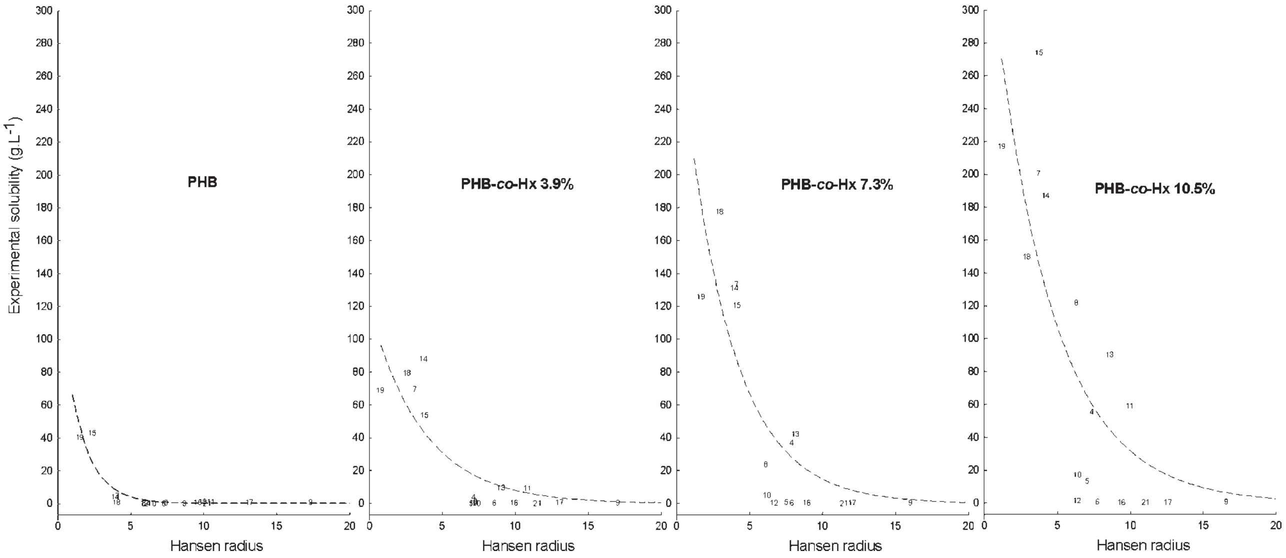

Figure 11. Representation of the experimental solubility of PHB-*co*-Hx (50°C) at different composition in Hx.

Each number refers to the solubility of a PHB-solvent system. The regression curves were established according to Eq. 12.

crystalline state is obtained by the destruction of this layer during polymer extraction.[28,33–35] According to the report of Lauzier et al., water is responsible for keeping the core of nascent PHB granules in a noncrystalline state.

The crystallizing rate is dependent on the kind of bacteria. Hahn et al.[36] find that, P(3HB) from recombinant *E. coli* and from *R. eutropha* (former name *Alcaligenes eutrophus*) have different degradation behavior with sodium hypochlorite. DSC test operated on these PHB showed that most of the polymer in *R. eutropha* was in a mobile amorphous state, whereas, the nascent PHB in *E. coli* strain was crystalline.[36] To eliminate the influence of this parameter, the PHB used in this study came from the same producer: Jiangsu Nantian Group. This polymer is obtained from the bacterial fermentation of *E. coli* strain. In such conditions, the crystallinities of the polymer samples are quite similar (Table 4), and so it is not a factor in this study.

PHB Copolymers

In this part, we will compare the influence on the solubility in the presence of a comonomer in the PHA composition. This study was based on the data obtained from three different PHB-*co*-Hx copolymers. Table 8 summarizes the properties of theses copolymers. By comparing, results obtained with PHB homopolymers and copolymers, it is obvious that the solubility behavior is totally different. First, the solubility is increasing with the Hx content of the polymer, Figure 11 shows this trend. On the other hand, some PHB-bad or low-solubility solvents, like acetic acid, pyridine, aniline, acetone, or dimethylformamide, lead to high solubility levels with PHB copolymers. Solubility of these solvent are comparable or higher than solubility of halogenated solvents such as chloroform, dichloromethane, or 1,2-dichloroethane. This behavior could be explained by the fact that the polymer solubility parameters are changing and become closer to these solvents. Judging from the large variations in the solubility behavior between PHB and PHB copolymers, the small difference in the crystallinity and in the molecular weight values could not be considered as predominant parameters (Table 8). By using the model of regression in Eq. 12, we obtained the correlation coefficients shown in Table 9. The comparisons of the results obtained by the 1-, 3-, and 4- parameters models show, as the work for homopolymer, that the best results are obtained with the 3-parameters Hansen model.

Then, by plotting the coefficients A_{Hx} and B_{Hx} from the Hansen parameters with the Hx content (%), Figure 12 is obtained. Then, by regressing these coefficients with the Hx content of the polymer, we could obtain correlations expressed in Eqs. 20 and 21:

$$A_{\mathrm{Hx}} = 10.76 + 100.6\ \exp\ (0.123(100X_{\mathrm{Hx}})), \quad R^2 = 0.878 \tag{20}$$

Table 9. Parameters of Eq. 12 for Different Hx Contents

	Hx, %			
	0 (M_{w} = 690,000)	3.9 (M_{w} = 967,000)	7.3 (M_{w} = 771,00)	10.5 (M_{w} = 427,000)
Hildebrand model				
R^2	0.191	0.416	0.502	0.464
A_{Hx}	18.4	65.9	146.4	192.8
B_{Hx}	0.630	0.569	0.529	0.440
Hansen model				
R^2	0.852	0.724	0.731	0.670
A_{Hx}	132.4	119.4	301.2	362.6
B_{Hx}	0.694	0.270	0.301	0.244
Beerbower model				
R^2	0.780	0.758	0.518	0.566
A_{Hx}	164.4	351.7	227.6	409.5
B_{Hx}	0.907	0.579	0.271	0.295

Hx refers to the PHB-*co*-Hx (T = 50°C).

$$B_{Hx} = 0.272 + 0.422\ \exp\left(-13.51(100\ {}_{n}X_{Hx})\right), \quad R^2 = 0.989 \tag{21}$$

Finally, we could express the solubility by the following formula in Eq. 22:

$$s = (10.76 + 100.6\ \exp(0.123(100\ X_{Hx}))\ \exp(-(0.272 + 0.422\ \exp(-13.51(100\ X_{Hx})))r) \tag{22}$$

Then, by joining the two parameters studied before, temperature and molecular weight, to Eq. 22 we obtained the following formula in Eq. 23:

$$s = (20.06 + 100.6\ \exp(12.3\ X_{Hx}) + 0.454\ \exp(0.063\ T) + 292.74\ \exp(-0.211\ M_W))\ \exp((-0.272 - 0.422\ \exp(-1351\ X_{Hx}))r) \tag{23}$$

The prediction obtained by Eq. 23 is compared with the experimental values in Figure 13. Although, the accuracy of this method is not very high especially for low solubility solvent–polymer system, but this method could give interesting results which could be applied in initial screening of solvents for solubilization and extraction purposes.

Conclusion

The method described in this article, demonstrated that using solubility parameters can lead to an evaluation of the solubility of PHB in various solvent systems. First, the polymer solubility parameters are evaluated by a novel method, which place the polymer in the solubility map as the barycentre of all the solvents. Second, the regression established between experimental solubility and solubility parameters can lead to an easy method for predicting solubility. Important parameters, which influence solubility, have been studied, and from the data collected some correlations have been made to take into account the impact of these parameters. The final correlation, which links solubility to the solubility radius (evaluated with solubility parameters), temperature, the polymer molecular weight, and the Hx content, have been tested on 232 experimental points. Results show that although accuracy of this method is not high, it gives interesting results to do a solvent selection before testing. Using this method coupling with their prices and low toxicity, solvents could be selected from their efficiency in solubilizing the polymer. This solvent screening method could also be very useful for the elimination of undesirable impurity, such as the elimination of residual endotoxin in PHAs that are intended for use in medical applications.

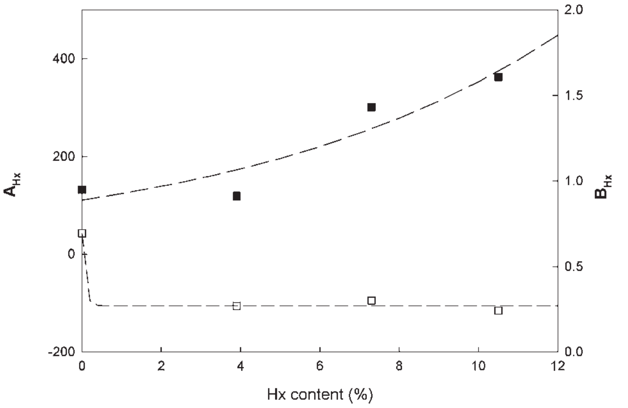

Figure 12. Variation of the regression coefficients A_{Hx} (□) and B_{Hx} (■) with the polymer molecular weight (operating temperature 50°C).

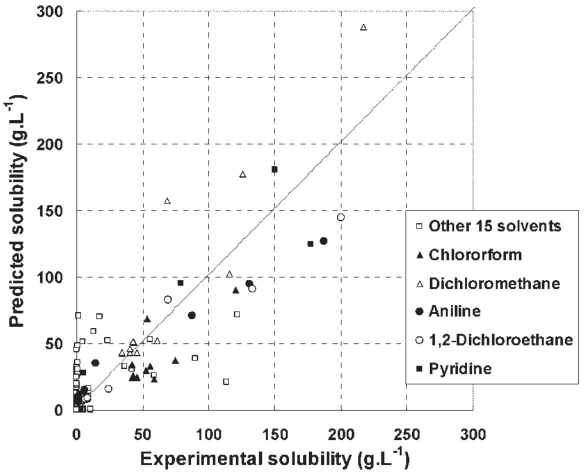

Figure 13. Predicted and experimental solubility values of 232 solvent–polymers systems with a polymer Hx content from 0 to 10.5%, a polymer molecular weight from 4500 to 1,300,000 and a temperature between 30 and 70°C.

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Notation

A = regression constant
B = regression constant
$C_0,\ldots,C_7$ = constants
D = regression constant
E_h = group molar attraction constant (J/mol)
F_d = group molar attraction constant ($J^{1/2}\ cm^{3/2}\ mol^{-1}$)
F_p = group molar attraction constant ($J^{1/2}\ cm^{3/2}\ mol^{-1}$)
R = solubility radius
T = temperature (K)
X = molar fraction
X_c = crystallinity rate

Greek letters

α = Activity coefficient
Δ = refer to a solvent
ΔH_m = polymer melting enthalpy (J/g)
ΔH_{PHB} = melting enthalpy of the 100% crystalline PHB (J/g)
δ_a = Beerbower Lewis acid interactions component ($MPa^{1/2}$)
δ_b = Beerbower Lewis base interactions component ($MPa^{1/2}$)
δ_t = Hildebrand total solubility parameter ($MPa^{1/2}$)
δ_λ = Prausnitz nonpolar cohesion parameter ($MPa^{1/2}$)

δ_τ = Prausnitz polar cohesion parameter (MPa$^{1/2}$)
δ_d = Hansen dispersion component (MPa$^{1/2}$)
δ_h = Hansen hydrogen bonding component (MPa$^{1/2}$)
δ_p = Hansen polar component (MPa$^{1/2}$)
Π = refer to a polymer

Subscripts

i = refer to the different solubility components
M = refer to a constant calculated in the molecular weight study
T = refer to a constant calculated in the temperature study

Superscripts

j = refer to a given solvent
k = refer to a given polymer

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