Compatible organic and natural solvent mixture of synthesising biodegradable polymeric nanoparticles

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Abstract. The Flory-Huggins model interaction explained the compatibility and extent of polymer dissolution in selected solvent mixtures via Hansen Solubility Parameters (HSP). Metastable zone where nucleation of NPs would start was determined by the solvent mixture – polymer – water interaction. Simulation results explained that the combination of acetone-chloroform (0.20:0.80) was better than acetone-ethyl lactate (0.40:0.60) for PCL solvation while ethyl lactate-dimethyl sulfoxide (0.60:0.40) was better for PLA solvation as compared to ethyl lactate-acetone (0.80:0.20). Nanoprecipitation with aqueous to organic volume ratio of 10 was used to prepare the biodegradable PCL nanoparticles for experimental validation. The organic phase was 1 g L−1 PCL in solvents or solvent mixtures and the antisolvent was deionized (DI) water. Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM) were used to examine the morphology and size of nanoparticles formed. Results showed that the acetone-chloroform with volume fraction of 0.20 to 0.80 was the best solvent mixture for PCL in producing NPs with the mean size less than 100 nm. Solvent mixture proved by numerical simulation and experimental validation, able to enhance the affinity of polymer (PCL or PLA) for water to produce nanoparticles with much smaller size.

1 Introduction

A big wheel in the development of monodispersed polymeric nanoparticles (NPs) is the inability to control the mixing processes required for their synthesis resulting in variable nanoparticle physicochemical properties [1, 2]. This biodegradable NPs have been used in therapeutic application, especially in drug delivery, which is a site-specific (targeted) delivery (Mahapatro & Singh, 2011). NPs has been prepared though several methods, such as emulsification-solvent evaporation, salting-out, dialysis, nanoprecipitation, and supercritical fluid technology or directly synt...
polymer behaviour, such as polymer concentration and molar mass of polymer in organic solution on the production of PLA nanoparticles by nanoprecipitation. High yields of small nanoparticles were obtained with polymers of lower molar mass.

In this present work, a novel combined organic solvent strategy was developed to formulate the nanoparticles, while the previous studies were only based on a single organic solvent. Organic solvent plays an important role in solvent displacement method. Since the most precipitation occurs in the mixed solvent systems, an inclusive co-relation principle for the solvent optimization is expected. In this context, Hansen Solubility Parameter (HSP) can be used to measure a distance between two molecules ($R_a$), where a model experiment was designed to explore the influence of mixed solvents on the nanoparticles synthesis [11, 12]. HSP is a parameter to determine the solubility of nanoparticle in a solvent dispersion. The smaller $R_a$, the more likely they are to be compatible. The strategy for mixed solvent selection is useful to choose optimal synthesis media for efficient biodegradable nanoparticles formation [12]. To fulfil this notion, three (3) main objectives made in this study were, (i) to design new engineered functional polymeric nanoparticle synthesis methods using a mixture of organic/natural solvents; (ii) to identify the optimum formulation parameters by using HSP; and (iii) to experimentally verify the attained numerical simulation using different physio-chemical analyses on the synthesised polymeric NPs.

2 Materials and methods

2.1 Chemicals

Poly-ε-caprolactone (PCL, $M_w = 14,000$ g mol$^{-1}$ with glass transition temperature of 60 °C) was purchased from Sigma-Aldrich (Malaysia). Acetone (analytical grade), chloroform (purity > 99.0%), ethyl lactate (purity > 99.0%) were supplied by Merk (Malaysia). The antisolvent phase was deionized water or demineralized water produced via ion exchange principle to remove the unwanted inorganic ions dissolved in water. The organic phase was a homogeneous solution of pure solvent or solvent mixture with $1$ g L$^{-1}$ (1000 ppm) of the polymer (PCL) in each solvent.

2.2 Experimental set-up and preparation of polymeric nanoparticles

The grinded PCL polymer powder was added into the solvent or solvent mixture and mixed well at 350 rpm for 5 minutes to form a homogenous organic solution. This solution was then dispersed into the aqueous phase to immediately perform a nanosuspension. A volume ratio between aqueous to organic phase ($V_{aq}/V_{or}$) was maintained at 10 by keeping a constant volume of aqueous phase. Fig. 1 illustrates the overall steps involved in preparing the polymeric nanoparticles by nanoprecipitation method. A rapid mutual diffusion of polymer from organic phase into organic / aqueous phase interface is applied in this precipitation method by chemical instability mechanism of stranded colloidal [5, 6]. Five different sets of organic solvent mixture, OR1 (main solvent) and OR2 (co-solvent) were prepared at different selective volume fraction, as summarised in Table 1 to Table 4.

![Fig. 1. Schematic diagram of nanoprecipitation procedures.](image)

2.3 Physico-chemical characterisation

2.3.1 Polymer dissolution and Atomic Force Microscope (AFM).
An initial analysis on the polymer dissolution has been done using different selected pure solvent, acetone and chloroform and a mix-solvent (acetone-chloroform). The required time to dissolve polymer was measured accordingly. Meanwhile, the surface topography and size of the nanoparticles were investigated using atomic force microscope (AFM). A sample drop of nanosuspension was deposited onto a microscope slide (22 mm x 22 mm). The dried sample was then tested using a SII Scino Instrument INC SPI 3800N Atomic Force Microscope (Failure Analysis Lab, School of Microelectronic Engineering, UniMAP) with “light” tapping mode by using tip to analyze the three-dimensional characteristics of the flat sample. The scanning speed used was 1.50 Hz with light angle of 45°. Tapping mode (dynamic AFM mode) operation considered as the best choice among contact mode, non-contact mode and tapping mode as it taps the surface with controlled velocity reported by Nanoscience Instruments (2019) able to improve the lateral resolution on soft and flat sample.

3 Results and discussion

3.1 Prediction of polymer-pure solvent and polymer-water interactions

The selection of organic solvent is essential prior to have a better polymer dissolution for forming a tunable size of polymeric nanoparticles. The polymer must be wholly dissolved in the organic solvent and miscible with water, which could be determined by the three-dimensional solubility parameters (δd, δp, δh) combined solubility parameters [13]. δd is the dispersion solubility parameter form London dispersion forces yielding to the presence of induced two molecules dipoles, δp is the polar solubility parameter resulted by Keesom forces at the presence of two permanent dipoles, and δh is the hydrogen bonding solubility parameter [11,12].

The polymer-solvent interaction can be predicted from a 2-D Bagley’s graph [14], in between a hydrogen bonding solubility parameter, δh to δp, where δp = (δd^2 + δh^2)^1/2. The best good solvents supposedly included within a circle of five δ-units radius of around the polymer [5, 6, 13]. Fig. 2 proves that water was located far apart from both solubility circles as expected, which supports the fact that water is not a solvent for polymer. The distance of solvents from the centre point of polymer PCL within the solubility circle limit increases in the following order: THF < Ac < CHCl3 < EL. Thus, THF shows highest affinity for PCL as it is located almost overlapping the central point of PCL, which means it can dissolve PCL very well.

Meanwhile, for PLA, the increasing order for distance of solvents from position of PLA within its solubility circle is as follows: EL < THF < DMSO < Ac. Thus, EL and THF are better solvents for PLA compared to DMSO and Ac. For a good solvent, polymer-solvent interactions are energetically favourable which allows polymer chain disentanglement that enhances solvation. In fact, a better interaction radius, R0 for PCL is 7.1 [11], while for PLA is 6.4 [12]. However, IPA and EtOH indicated D > 7.1 and D > 6.4, which revealed as the bad solvents for PCL and PLA, respectively. This resulted no polymer dissolution and merely favor to polymer-polymer self-interactions, hence the polymer coils contract and globule formation. These graphical observations apparently were well correlated to our previous work [5, 6]. Therefore, THF, Ac and EL solvents will be selected for further mixed-solvent interactions study.
Fig. 2. Bagley’s two-dimensional graph of the partial solubility parameters of the pure solvents with respect to the partial solubility parameters determined for PCL and PLA (line (+) = solubility circle limit for PCL; line (-) = solubility circle limit for PLA).

3.2 Prediction of solvent mixture – water interaction

The selection of penetrating solvent will have a profound effect on polymer dissolution [4]. The selected solvent mixture thus must be able to cause polymer solvation and must be miscible with water. The combined solubility parameter, $\Delta \delta_{m}$ as shown in equation (1) was used to predict the interaction between solvent mixture and water, where $w$ represents water and $m$ denotes solvent mixture.

$$\Delta \delta_{m} = [(\delta_{d,w} - \delta_{d,m})^2 + (\delta_{h,w} - \delta_{h,m})^2 + (\delta_{p,w} - \delta_{p,m})^2]^{1/2}$$ (1)

For solvent mixture of Ac (OR1) and EL (OR2), the $\Delta \delta_{m}$ value from Table 1 shows an increasing trend with increasing volume fraction of Ac in solvent mixture. This means EL has higher affinity for water as compared to Ac. This is due to hydrogen bonding interaction of EL is more alike to water as compared to Ac, thus EL is more miscible with water. Meanwhile, for solvent mixture Ac (OR1) and CHCl3 (OR2), the $\Delta \delta_{w}$ value from Table 2 indicated a decreasing trend with increasing concentration of Ac in Ac-CHCl3 solvent mixture. This indicates CHCl3 is less compatible with water as compared to Ac.

Meanwhile, solvent mixture of EL (OR1) and Ac (OR2) used for PLA as the polymer was summarised in Table 3. The $\Delta \delta_{w}$ value shows reversing order trend as compared to Ac (OR1) and EL (OR2) pairing for PCL in Table 1 as EL is the main solvent for PLA while Ac is the main solvent for PCL. For solvent mixture EL (OR1) and DMSO (OR2), it shows an increasing $\Delta \delta_{w}$ value with increasing EL in the mixture, as depicted in Table 4. This is due to higher polarity of EL that leads to a similar behavior as water with its hydrophilic molecule.

By comparing the $\Delta \delta_{w}$ value for four solvents, the $\Delta \delta_{w}$ value increases in the following order: DMSO < EL < Ac < CHCl3. This proves that DMSO favors the formation of smaller nanoparticles in terms of affinity for water because the smaller the value for $\Delta \delta_{w}$, the smaller the cohesion energy difference between selected solvent and water, the higher the affinity of selected solvent for water and the higher its solubility into aqueous phase (water) [5, 6].

Table 1. OR1 = Ac, OR2 = EL and $p$ = PCL. The partial solubility parameters for solvent mixture OR1 (main solvent) and OR2 (cosolvent), $\delta_{d,OR1}$, $\delta_{h,OR1}$, $\delta_{p,OR1}$ and $\delta_{d,OR2}$, $\delta_{h,OR2}$, $\delta_{p,OR2}$, the combined solubility parameters, $\Delta \delta_{w}$, and $\Delta \delta_{m}$, $\Delta \delta_{w}$, and $\Delta \delta_{m}$, $\phi$ and $\phi$ = polymer (PCL or PLA), $m$ = solvent mixture, $w$ = water, distance $d$ between $m$ and $p$ in solubility circle and distance $D$ between $m$ and $p$ in Hansen solubility space, molar volume of solvent mixture, $V_{solvent}$ mixture and solvent mixture - polymer interaction parameters, $\chi$ solvent mixture - polymer. Unit of solubility parameters = (1cm$^3$)mol$^{-1}$, unit of molar volume of solvent mixture = cm$^3$mol$^{-1}$. Row with bold font denotes the best combinations of solvent mixture.

| $\phi$OR1 | $\phi$OR2 | $\delta_{d,OR1,OR2}$ | $\delta_{h,OR1,OR2}$ | $\delta_{p,OR1,OR2}$ | $\Delta \delta_{w}$ | $\Delta \delta_{m}$ | $\Delta \delta_{w}$ | $\Delta \delta_{m}$ | $d$ | $D$ | $V_{solvent}$ mixture | $\chi$ solvent mixture - polymer |
|----------|----------|----------------------|----------------------|----------------------|-------------------|-------------------|-------------------|-------------------|-----|-----|---------------------|-----------------------------|
| 0.00     | 1.00     | 15.95                | 7.57                 | 12.48                | 17.66             | 5.12              | 32.36             | 4.18              | 5.4 | 114.69 | 1.22                |
| 0.20     | 0.80     | 15.85                | 8.14                 | 11.38                | 17.82             | 4.68              | 32.69             | 3.08              | 5.1 | 106.53 | 0.94                |
| 0.40     | 0.60     | 15.75                | 8.70                 | 10.27                | 18.00             | 4.55              | 37.07             | 2.00              | 5.0 | 98.37  | 0.82                |
| 0.60     | 0.40     | 15.66                | 9.27                 | 9.17                 | 18.19             | 4.75              | 33.49             | 1.02              | 5.3 | 90.21  | 0.82                |
| 0.80     | 0.20     | 15.56                | 9.83                 | 8.06                 | 18.41             | 5.24              | 33.96             | 0.78              | 5.8 | 82.05  | 0.91                |
| 1.00     | 0.00     | 15.46                | 10.40                | 6.96                 | 18.63             | 5.96              | 34.66             | 1.65              | 6.5 | 73.89  | 1.06                |

Table 2. OR1 = Ac, OR2 = CHCl3 and $p$ = PCL.

| $\phi$OR1 | $\phi$OR2 | $\delta_{d,OR1,OR2}$ | $\delta_{h,OR1,OR2}$ | $\delta_{p,OR1,OR2}$ | $\Delta \delta_{w}$ | $\Delta \delta_{m}$ | $\Delta \delta_{w}$ | $\Delta \delta_{m}$ | $d$ | $D$ | $V_{solvent}$ mixture | $\chi$ mixed solvent - polymer |
|----------|----------|----------------------|----------------------|----------------------|-------------------|-------------------|-------------------|-------------------|-----|-----|---------------------|-----------------------------|
| 0.00     | 1.00     | 17.80                | 5.70                 | 18.07                | 3.21              | 40.45             | 2.6               | 3.5               | 80.12 | 0.33 |                      |                             |
| 0.20     | 0.80     | 17.33                | 4.56                 | 5.95                 | 17.92             | 2.38              | 39.20             | 2.4               | 2.5 | 78.88  | 0.18                |
| 0.40     | 0.60     | 16.86                | 6.02                 | 6.20                 | 17.91             | 2.43              | 37.98             | 2.1               | 2.4 | 77.63  | 0.18                |
| 0.60     | 0.40     | 16.40                | 7.48                 | 6.46                 | 18.02             | 3.31              | 36.77             | 1.9               | 3.5 | 76.38  | 0.34                |
| 0.80     | 0.20     | 15.93                | 8.94                 | 6.71                 | 18.27             | 4.56              | 35.60             | 1.7               | 4.9 | 75.14  | 0.63                |
| 1.00     | 0.00     | 15.46                | 10.40                | 6.96                 | 18.63             | 5.96              | 34.46             | 1.7               | 6.5 | 73.89  | 1.06                |

Table 3. OR1 = EL, OR2 = Ac and $p$ = PLA.
The combined solubility parameter $\Delta \delta_{p,m}$ shown in equation (2) was calculated using new combined HSP corresponding to the different volume fraction of solvent mixture by utilizing mixing rules that have been established by Hansen [12] where $p$ and $m$ represent polymer (PCL or PLA) and solvent mixture respectively.

$$\Delta \delta_{p,m} = [(\delta_{p,p} - \delta_{p,m})^2 + (\delta_{p,p} - \delta_{p,m})^2 + (\delta_{p,p} - \delta_{m,m})^2]^{1/2}$$

The $\Delta \delta_{p,m}$ value for Ac-EL combination as shown in Table 1 and Ac-CHCl3 combination as shown in Table 2 increases in the order as follows: Ac-EL (0.40:0.60) < EL < Ac and Ac-CHCl3 (0.20:0.80) < CHCl3 < Ac. This indicates that Ac-EL (0.40:0.60) favors the mixing of PCL compared to pure EL and pure Ac while Ac-CHCl3 (0.20:0.80) is the most favourable mixing ratio for PCL to dissolve compared to pure CHCl3 and pure Ac. This can be due to the smaller the value of $\Delta \delta_{p,m}$, the higher its compatibility with polymers. The combined solubility parameter, $\Delta \delta_{p,m}$ also has been calculated for EL-Ac-PLA and EL-DMSO-PLA combinations as shown in Table 3 and Table 4 in order to investigate how compatible the PLA is in the solvent mixture. The increasing $\Delta \delta_{p,m}$ value trend is as follows for EL-Ac and EL-DMSO: EL-Ac (0.80:0.20) < EL < Ac and EL-DMSO (0.60:0.40) < EL < DMSO. The smallest value of $\Delta \delta_{p,m}$ indicates PLA dissolves well in the solvent.

Besides, the $D$-value, which is about the distance between a solvent mixture and polymer in the solubility space was also calculated using equation (3):

$$D = [4(\delta_{p,p} - \delta_{p,m})^2 + (\delta_{p,p} - \delta_{p,m})^2 + (\delta_{p,p} - \delta_{m,m})^2]^{1/2}$$

For PCL, the $R_0$ for PCL is 7.1. This means the $D$-value of solvent mixture with PCL must be less than 7.1 to allow PCL solvation occurs. As expected, all the volume fractions combination for both Ac-EL and Ac-CHCl3 are solvents for PCL as $D < 7.1$, which in agreement with the graphical interpretation as discussed in section 3.2 using base graph.

For Ac-EL, the $D$-value increases as follows: Ac-EL (0.40:0.60) < EL < Ac while for Ac-CHCl3, the $D$-value increases as follows: Ac-CHCl3 (0.20:0.80) < CHCl3 < Ac. The nearer the $D$-value to the PCL, the better the interaction of the solvent with the polymer, the better it is as solvent for PCL. For PLA, the interaction radius is 6.4. The $D$-value for EL-Ac increases with the following sequence: EL-Ac (0.80:0.20) < EL < Ac while EL-DMSO is as follows: EL-DMSO (0.60:0.40) < EL < DMSO.

Based on the results using equation (2) and equation (3), it shows a direct relationship between $\Delta \delta_{p,m}$ and $D$. The combined solubility parameter $\Delta \delta_{p,m}$ and $D$ both show same trend of increasing order for all combinations of solvent mixture with two polymers (PCL and PLA) that denotes the smaller the $\Delta \delta_{p,m}$ higher the compatibility between solvent mixture and polymer, and thus the smaller the $D$ in Hansen sphere solubility space, the higher the solubility of polymer in solvent mixture which favours polymer dissolution in solvent mixture.

### Table 4. OR1 = EL, OR2 = DMSO and $p = PLA.$

| OR1 | OR2 | $\delta_{p,OR1}$ | $\delta_{p,OR2}$ | $\delta_{p,OR1}$ | $\delta_{p,OR2}$ | $\Delta \delta_{p,m}$ | $\Delta \delta_{m}$ | $\Delta \delta_{p,m}$ | $\Delta \delta_{m}$ | $D$ | $V_{solvent}$ | $Z_{solvent}$ |
|-----|-----|------------------|------------------|------------------|------------------|----------------------|------------------|----------------------|------------------|-----|----------------|------------------|
| 0.00 | 0.40 | 15.75            | 8.70             | 10.27            | 18.00            | 2.59                 | 33.07            | 2.6                   | 4.1               | 98.37 | 0.27           | 0.00              |
| 0.80 | 0.20 | 15.85            | 8.14             | 11.38            | 17.82            | 2.39                 | 32.69            | 2.3                   | 3.9               | 106.53 | 0.25           | 0.00              |
| 1.00 | 0.00 | 15.95            | 7.57             | 12.48            | 17.66            | 2.80                 | 32.36            | 2.6                   | 4.0               | 114.69 | 0.36           | 0.00              |

### Table 3. OR1 = EL, OR2 = CHCl3 and $p = PLA.$

| OR1 | OR2 | $\delta_{p,OR1}$ | $\delta_{p,OR2}$ | $\delta_{p,OR1}$ | $\delta_{p,OR2}$ | $\Delta \delta_{p,m}$ | $\Delta \delta_{m}$ | $\Delta \delta_{p,m}$ | $\Delta \delta_{m}$ | $D$ | $V_{solvent}$ | $Z_{solvent}$ |
|-----|-----|------------------|------------------|------------------|------------------|----------------------|------------------|----------------------|------------------|-----|----------------|------------------|
| 0.00 | 0.40 | 16.91            | 11.07            | 11.57            | 20.21            | 1.55                 | 30.69            | 0.2                   | 2.0               | 97.34 | 0.09           | 0.00              |
| 0.80 | 0.20 | 16.43            | 9.32             | 12.02            | 18.89            | 1.27                 | 31.48            | 1.3                   | 2.4               | 106.02 | 0.07           | 0.00              |
| 1.00 | 0.00 | 15.95            | 7.57             | 12.48            | 17.66            | 2.80                 | 32.36            | 2.6                   | 4.0               | 114.69 | 0.36           | 0.00              |
Graphical analysis has also been carried out the further validate the simulation. The $d$-value (shown in equation (4)) is the distance between a solvent mixture and polymer in 2D solubility graph has been calculated prior to be plotted onto graph. The $d$-value takes in consideration of $\delta_v$., volume-dependent solubility parameter, which summing up the two terms, $\delta_d$ and $\delta_p$, in order to transform it into two parameter concept graph with $\delta_v$ plotted against $\delta_v$.

$$d = \sqrt{(\delta_v,M - \delta_v,P)^2 + (\delta_h,M - \delta_h,P)^2}$$

(4)

where subscript $M$ represents mixture of combined solvent-water and subscript $p$ represents polymer (PCL or PLA).

The $d$-value is the distance of solvent and polymer in 2D-graph. For Ac-EL, $d$-value increases in following order: Ac < Ac-EL (0.40:0.60) < EL and for Ac-CHCl3, $d$-value increases as follows: Ac < Ac-CHCl3 (0.20:0.80) < CHCl3. This means Ac was lied nearest to the PCL in both solvent mixture combinations. The graphical analysis has been done for Ac-EL and Ac-CHCl3 as shown in Figure 3 (a) and Figure 3 (b), which in agreement with the $d$-value calculated. Both figures show that Ac is nearest to the central point of PCL within the solubility limit circle. Based on the numerical data shown in Table 3, for EL-Ac, $d$-value increases as follows: EL-Ac (0.80:0.20) < EL < Ac. In Table 4, the $d$-value for EL-DMSO increases by the following order: EL-DMSO (0.60:0.40) < EL-DMSO (0.80:0.20) < EL < Ac. Based on observation, both $d$-values for solvent mixture - polymer again prove that Ac is the main solvent for PCL and EL is the main solvent for PLA as both possesses nearer distance in 2-D graph compared to their respective co-solvents.

To analyze the exact interaction between solvent mixture and polymer, solvent mixture - polymer interaction parameter, $\chi$ solvent mixture-polymer, is calculated. It serves as a universal unitless quantity parameter as it indicates the extent of solubility of polymer in the solvent. If $\chi$ solvent mixture-polymer < 0.5, polymer is soluble in that solvent or solvent mixture over entire concentration range. Conversely, if for $\chi$ solvent mixture-polymer > 0.5, polymer is hardly dissolve. Equation for $\chi$ solvent mixture-polymer, as shown below:

$$\chi_{solvent\ mixture-polymer} = \frac{V_{solvent\ mixture}}{RT} \left[ (\delta_{h,n} - \delta_{d,p})^2 + (\delta_{h,m} - \delta_{d,p})^2 + (\delta_{h,n} - \delta_{d,m})^2 \right]$$

(5)

where $V_{solvent\ mixture}$ is the molar volume of solvent mixture, $R$ is universal gas constant (8314 Jkmol⁻¹), $T$ is the absolute temperature (K).

**Fig 3.** Summary of Bagley's 2D-graph of the partial solubility parameters of the solvent mixtures (OR1 – OR2) ; (a) Ac – EL, (b) Ac – CHCl3, (c) EL – Ac, and (d) EL – DMSO, with respect to the partial solubility parameters determined for both PCL and PLA, respectively. The solubility circle shown by line (-) has a radius of 5 $\delta$-units.
The molar volume of solvent mixture, $V_{\text{solvent mixture}}$ increases in the following succession: Ac < Ac-EL (0.40:0.60) < DMSO and the $\chi_{\text{solvent mixture - polymer}}$ increases as follows: Ac < Ac-EL (0.40:0.60) < EL. This shows that molar volume and $\chi_{\text{solvent mixture - polymer}}$ is correlated although $\chi_{\text{solvent mixture - polymer}}$ for Ac-EL solvent mixture all are larger than 0.5 that implies the solubility of such combination might not be so good as the rate is diffusion rate for PCL into the solvent slow. In Table 2, the $V_{\text{solvent mixture}}$ for Ac-CHCl$_3$ increases as the following: Ac < Ac-CHCl$_3$ (0.20:0.80) < CHCl$_3$ but the $\chi_{\text{solvent mixture - polymer}}$ increases as follows: Ac-CHCl$_3$ (0.20:0.80) < CHCl$_3$ < Ac. The value of $\chi_{\text{solvent mixture - polymer}}$ for acetone is far from desired range of 0.5, which is 1.06 that means the polymer dissolution rate is slow in acetone and that might be takes much longer time than CHCl$_3$ and Ac-CHCl$_3$ (0.20:0.80) to dissolve PCL. For PLA, in Table 3, the trend of $V_{\text{solvent mixture}}$ for EL-AC increases as follows: Ac < EL (0.80-0.20) < EL < Ac meanwhile in Table 4, $V_{\text{solvent mixture}}$ for EL-DMSO increases in the order of DMSO < 0.60:0.40 < EL and $\chi_{\text{solvent mixture-polymer}}$ increases in the order of EL-DMSO (0.60:0.40) < EL < DMSO. Based on the value obtained, DMSO takes long time to dissolve PLA as the $\chi_{\text{solvent mixture-polymer}}$ > 0.5, which is 1.35. If using EL alone, polymer dissolution still can be occurred at fast rate but by utilizing combination of both EL and DMSO with ratio 0.60 to 0.40, the $\chi_{\text{solvent mixture-polymer}}$ value decreases drastically to only 0.09, which implies very fast PLA dissolution in this combination.

3.4 Selection of the best volume fraction for each solvent mixture with polymer

The size of NPs formed not only relying on the interaction of solvent mixture with polymer in organic phase but also depends on how compatible the organic phase is with the aqueous phase as both hydrophobic polymer (PCL and PLA) cannot dissolve readily in water. Thus, combined solubility parameter, $\Delta \delta_{\text{m}}$, is the conflict point that should be concerned in choosing the best volume fraction combination. PCL and PLA are both hydrophobic polymers, but PLA is more polar than PCL thus shows higher compatibility with aqueous phase (water) [5, 6].

Thus, for PCL, the relationship between $\Delta \delta_{\text{m}}$ and D with $\Delta \delta_{\text{m}}$ was in an opposite trend with the higher compatibility of PCL with solvent mixture. This trend is clearly shown for pure solvent volume fraction in Table 1 and Table 2. Thus, the main solvent (Ac) assists the solubility of polymer in water and the cosolvent (EL or CHCl$_3$) enhances polymer solvation in organic phase. Thus, for Ac-EL combination, volume fraction of 0.40 to 0.60 has been chosen as the best combination while for Ac-CHCl$_3$, Ac-CHCl$_3$ (0.20:0.80) is the best volume fraction combination. Between both types of solvent mixture combination, Ac-CHCl$_3$ is better than Ac-EL (0.40:0.60) in terms of polymer compatibility and polymer diffusion rate as rate affects efficiency of dissolution.

Meanwhile, for PLA, due to its polarity, $\Delta \delta_{\text{m}}$ and D values as compared to cosolvent (Ac) but not for DMSO as DMSO has higher polarity then EL to water but lower similarity of HSP with PLA that allows DMSO to be more compatible with water as compared to EL. Although DMSO is more compatible with water than EL it cannot be considered as the main solvent as the $\chi_{\text{solvent mixture-polymer}}$ is 1.35, which is too much bigger than 0.5 that affects solubility of polymer alone. By numerical simulation, EL-Ac (0.80 – 0.20) and EL-DMSO (0.60 – 0.40) are the best volume fraction combination for each type of solvent mixture. By comparison, EL-DMSO (0.60:0.40) favours formation of smaller PLA NPs than EL-Ac (0.80 – 0.20) as the cosolvent, DMSO possesses good compatibility with water and very similar dispersion force with PLA that enhances polymer dissolution and NPs formation with the existence of EL, as a main solvent.

3.5 Prediction of solvent mixture – polymer – water interaction

Figure 3 (a) and Figure 3 (b) indicate the Ac-EL-PCL-water interaction and Ac-CHCl$_3$-PCL-water interaction respectively. The solubility circle around the PCL at this stage acts as the solubility cloud point for which nucleation occurs. Any point does not lie on the solubility cloud point means crystallization cannot occur and nanoparticles cannot be formed. This is the metastable zone which is a zone between the solubility curve (clear points) and the metastable limit curve (cloud point). This means solution that is not lie on the solubility cloud point is in undersaturated state and no nanoparticles will be formed as it is still dissolve in the solution. Based on the graph in Figure 3 (a), the point of 89 vol% Ac-EL (0.40:0.60) with 11 vol% water lies on the solubility cloud point, as in Figure 3(b), the point 76 vol% Ac-CHCl$_3$ (0.20:0.80) with 24 vol% water lies on the solubility cloud point. These are the points where nanoprecipitation starts, and crystal (nanoparticles) will start to grow. Ac-CHCl$_3$ is more diluted polymer solution as compared to Ac-EL, leads to smaller nanoparticles.

On the other hand, Figure 3 (c) and Figure 3 (d) show the EL-Ac-PCL-water interaction and EL-DMSO-PLA-water interaction respectively. From the graph in Figure 3 (a), nucleation will occur when 77 vol% EL-Ac (0.80:0.20) with 23 vol% water exists in the solution. Meanwhile, for EL-DMSO, nanoprecipitation starts at point 79 vol% EL-DMSO (0.60:0.40) with 21 vol% water. By comparing the behaviour for both polymer, PLA tends to precipitate in a more diluted
polymer solution due to its polarity that will limit crystal growth and producing smaller particle size as compared to PCL, which in agreement with Othman et al. [5, 6].

3.6 Experimental validation

3.6.1 Polymer dissolution in the selected solvent mixture.

Based on Table 5 indicates the average dissolution time taken by PCL at room temperature increases in the following order: CHCl₃ < Ac-CHCl₃ (0.20:0.80) < Ac. This shows agreement results with the numerical simulation as aforementioned discussed, where Ac is a polar molecule and needs the longest time to dissolve PCL. CHCl₃ is a non-polar molecule and as expected, took shortest time to allow PCL solvation. Ac-CHCl₃ with volume fraction (0.20:0.80) is a final selected solvent ratio to prepare Ac-CHCl₃ as it can mix well with polymer and with presence of Ac, it enhances its affinity for water to produce smaller sized nanoparticles.

PCL dissolution in EL and Ac-EL have not been shown in Table 1 as both cannot cause PCL solvation at room temperature. Heating process was carried out by using hot plate until 100 °C for PCL to be dissolved in pure EL or Ac-EL (0.40:0.60). This is due to diffusion rate of EL and Ac-EL (0.40:0.60) is very slow, which can be seen clearly in Table 1 that the χsolvent mixture–polymer Values of both EL and Ac-EL are more than 0.5, which are 1.22 and 0.82 respectively. Although, the χsolvent mixture–polymer Value of Ac was more than 0.5, which is 1.06, but due to the of δₘ value of Ac is quite similar to the δₘ value of PCL, “like seeks like” theory applied for this chemical force similarity and thus PCL can be dissolved in Ac at room temperature. This PCL dissolution test once again proves that Ac is the main solvent besides showing that CHCl₃ acts as a cosolvent to enhance solubility of PCL in organic phase by combination Ac-CHCl₃ (0.20:0.80). Heating process which enhances PCL dissolution in EL and Ac-EL (0.40:0.60) proves that χsolvent mixture–polymer is inversely proportional to temperature. By increasing the temperature of the organic phase solution, the χsolvent mixture–polymer value will be decreased, and PCL solvation happens.

Table 5. Average dissolution time taken by PCL in relevant solvents.

| Pure solvent/ solvent mixture | Average dissolution time (min) |
|------------------------------|--------------------------------|
| Acetone                      | 2.587 ± 0.384                  |
| Chloroform                   | 0.473 ± 0.046                  |
| Acetone-Chloroform           | 3.6 ± 0.079                    |

3.6.2 Particle size analysis of polymeric nanoparticles.

The three-dimensional (3D) view and top view images of PCL nanoparticles have been investigated using atomic force microscopy (AFM). Figure 4 proves that PCL the NPs formed by using Ac-CHCl₃ is the best organic phase for PCL as the size of particles from surface topography and top view show the smallest and the dispersion of NPs is the most uniform as compared to others. Thus, indicated CHCl₃ is more compatible to PCL, rather than Ac which enable uniform dispersion of NPs in nanosuspension. For EL, due to low compatibility with PCL, it shows the largest size of NPs among the solvent/solvent mixture. With the existence of Ac as main solvent in Ac-EL, due to the very small molar volume of Ac as compared to EL which promotes solubility, it enhances the PCL affinity and dissolution that enables more uniform and smaller size of NPs formed. Apart from smallest NPs size (62.7 nm) obtained for Ac-CHCl₃ as a mixed- solvent, Ac-EL mixture showed bigger size with 175.9 nm, pure solvent Ac at 111.8 nm, CHCl₃ with 137.3 nm and EL with 251.8 nm.
Fig 4. AFM images of PCL nanoparticles using Ac-CHCl$_3$(0.20:0.80) as organic solvent mixture: (a) 3D-view; (b) top view.

4 Conclusions

The numerical simulation and experimental data were well proven that solvent mixture able to enhance dissolution of polymer to produce nanoparticles with smaller size. To form solvent mixture, mixing rules have been established by Hansen for even complicated solvent combinations. It is done by summing up simple volume fraction multiplied by the solubility parameter values to produce new HSP that comprised of the 3 individual parts: dispersion force, polar force, and hydrogen bonding force interactions. Flory-Huggins interaction parameter, $\chi_{\text{solvent mixture-polymern}}$, is measure of the polymer-solvent interaction energy. It also indirectly giving information about the size and pattern of polymer coil in a solvent. It also takes in consider the importance of molar volume in calculation. Although molecular volume is an important factor for effective mixing, even always described as forth parameter in solubility parameter but it cannot be included in new composite solubility parameter as it is not a cause via thermodynamic considerations on which HSP are based on. It is rather a kinetic effect of diffusion rate that important in kinetic phenomena instead. By comparing the solvent mixture pairing results, for PCL, Ac-CHCl$_3$ is a better choice to act as organic phase for PCL than Ac-EL as its $\chi_{\text{combined solvent-polymern}}$ is in the range less than 0.5 as compared to Ac-EL, which is 0.82, greater than good solvent interaction limit. For PLA, EL-DMSO pairing is significantly better than EL-Ac as the $\chi_{\text{combined solvent-polymern}}$ value is as low as 0.09, which means polymer is favourable to be in contact with this solvent mixture with EL-DMSO ratio at 0.60:0.40. This shows a smaller size of polymeric NPs could be tuned by theoretically investigating the right mix-organic solvent initial solubility-capability with both water and polymer.

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