Dissolution of Condensed Tannin Powder-based Polyphenolic Compound in Water-Glycerol-Acid Solution

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Dissolution of polyphenolic compounds from condensed tannins powder from wattle species was carried out using water-glycerol mixtures and sulfuric acid (H_2SO_4) as a catalyst. The study focused on parameters that can be adjusted to maximize the dissolution. The parameters of the dissolution process (mass of glycerol, mass of tannin powder, temperature and stirring time) were screened using a one factor at a time (OFAT) technique, while the optimum conditions were obtained using response surface methodology (RSM). Effects of the mass of glycerol, mass of tannin powder, temperature, and stirring time used on the percentage of dissolved tannin residue was apparent. The amount of undissolved tannin was used as the direct measurement in this study since there is no established method available to test tannin dissolution in water-glycerol-acid solution. The result from RSM based on 30 experimental sets showed that the lowest undissolved tannin powder value was 10% when 75 grams of tannin powder was mixed with 13.56 grams of glycerol, 86.44 grams of water, and 1.00 grams of sulphuric acid, at 75 °C temperature and 44.13 minutes stirring time.

Keywords: Polyphenolic compounds; Water-glycerol; H_2SO_4; Condensed tannin; Dissolved tannin; Dissolution parameters

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INTRODUCTION

Tannins can be classified as natural polyphenolic compounds that are present in large concentrations in wood barks (Khanbabaee and van Ree 2001; Bertaud *et al.* 2012). Frutos *et al.* (2004) classified tannin into two classes, namely the hydrolysable and condensed tannins. Condensed tannins have a condensed chemical nature and are soluble in water (Ashok and Upadhyaya 2012). They are able to form bonds with other substances (Bertaud *et al.* 2012) and have been used as the source of wood adhesives. Condensed tannins or proanthocyanidins are phenolic compounds consisting of several types of flavan-3-ols and flavan-3,4-diols; they are polymers that are linked by carbon-carbon bonds between flavanols subunits (Schofield *et al.* 2001; Waghorn and McNabb 2003). Many studies have investigated the replacement of phenol with tannins in phenol-formaldehyde-type adhesives (Zhu *et al.* 2012; Feng *et al.* 2015; Li *et al.* 2016; Anwer and Naguib 2017; Lan *et al.* 2017; Li *et al.* 2018; Missio *et al.* 2018). Tannin powder has been traditionally dissolved in phenol for reactivation. Samil *et al.* (2005) were able to dissolve 90% w/w of

the condensed tannin in phenol with the presence of sulfuric and hydrochloric acids (4 to 5 %) at 160 °C. However, phenol is known for its toxicity and carcinogenic properties. A number of studies had substituted phenol with other solvents for dissolving tannin. One of the initial trials to replace phenol was using water (Hussein *et al.* 2011). They produced tannin suspension with coagulated tannin powder. Another trial was performed by Cardona and Sultan (2015) in which they dissolved up to 40% w/w tannin powder in glycerol-acid solution at 80 °C for 45 min. However, the formed product was highly viscous and sticky, which possibly reduced its pliability. Also, no optimization work was conducted by Cardona and Sultan (2015).

Glycerol, which is non-toxic and biodegradable, serves as an alternative green solvent (Bosart and Snoddy 1927; Wolfson *et al.* 2007; Gu and Jerome 2010; Nemati *et al.* 2016; Wolfson *et al.* 2016; Pagliaro 2017; Skulcova *et al.* 2018). These properties make it a good solvent to replace phenol in the tannin dissolution process. Shrivastava *et al.* (2017) described that tannins from plants have the capability to bind with the glycerol. The presence of multiple hydroxyl groups in glycerol make it similar to water and simple aliphatic alcohols, such that it is categorized as a good solvent (Cardona and Sultan 2015). The reaction can be accelerated by the addition of an acid catalyst (Samil *et al.* 2005; Cardona and Sultan 2015). The presence of hydroxyl groups on tannins allowed the formation of stable cross-linking between polyphenolic compounds with macromolecules such as polyols (glycerol) (Khanbabaee and van Ree 2001; Ashok and Upadhyaya 2012; Shrivastava *et al.* 2017). Shrivastava *et al.* (2017) claimed that tannins can be incorporated into glycerol to form a homogenous solution. Catechin is a flavan-3-ol (or flavanol), which is a type of natural polyphenolic compound (Bernatoniene and Kopustinskiene 2018). The repeating units of catechins formed condensed tannin, with at least two linked units of catechins (Khanbabaee and van Ree 2001; Ashok and Upadhyaya 2012; Shrivastava *et al.* 2017). There are multiple hydroxyl groups in the catechin chemical structure that make tannin easily bond to glycerol. Glycerol, which is a polyol, contain sufficient free oxygen molecules to form strong hydrogen bonds with the hydroxyl groups in the catechin (Shrivastava *et al.* 2017).

Glycerol is a natural additive to tannin and is able to plastice the base resin (Cardona and Sultan 2015). However, using a high amount of glycerol in dissolving tannin powder caused: 1) a reduction in the amount of tannin that could be dissolved, as shown by Cardona and Sultan (2015). Since, tannin is required to reduce the brittleness properties of phenol formaldehyde resin (Samil *et al.* 2005; Jahanshaei *et al.* 2012; Cardona and Sultan 2015), it is important to be able to increase the amount of tannin powder that could be dissolved in a solvent. 2) The produced dissolved tannin is highly viscous. To produce laminating material, it is required to produce resin with the viscosity ranging from 400 to 600 cP, as suggested by the industry (Gillern *et al.* 1981) in order to produce pliable resin and good laminating material. It has been found that the use of highly viscous dissolved tannin leads to the production of high viscous dissolved tannin phenol formaldehyde resin, which is not suitable as a laminating material. Addition of water to glycerol helps in reducing the viscosity of the glycerol. The interaction between polar water [solvent] and polar glycerol [solute] brings about dissolution (Towey and Dougan 2012). Glycerol and water act as co-solvent in this work; their behaviors and properties can best be described using the colligative solutions' properties. The binary mixture of glycerol and water and their respective properties had been described in many literatures including Segur and Oberstar (1951), Dashnau *et al.* (2006), and Hansen (2007). The interaction between the polar solvent and polar solute follows the rule of thumb 'like dissolve like' as described by

Hansen (2007). Therefore, the water-glycerol solution prepares a medium as a polar solvent to dissolve polar catechin (Lai *et al.* 2008) in the condensed tannin at suitable temperature and time (Cardona and Sultan 2015).

The aim of this project was to investigate the dissolution of tannin powder in water-glycerol acid solution to produce a less viscous tannin solution, which will be more pliable and will tend to minimize the undissolved tannin powder that remains. This work focused on the optimization of the dissolution process and parameters to minimize the use of glycerol and maximize the dissolution of tannin. A response surface methodology (RSM) program from Design Expert was used to find the best conditions to dissolve tannin. The extended part of the project will include the synthesis of tannin-based laminate composite that will act as a surface protector. Investigating the tannin dissolution is important to ensure the introduction of defects into the structure is minimized.

EXPERIMENTAL

Tannin extracts from the bark of the black wattle (Acacia mearnsii L.) B345 Bondtite were provided by Bondtite Adhesives (Pty) Limited (Pietermaritzburg, South Africa). Deionized water and 97% Glycerol were used as the main and co-solvents, and sulphuric acid was used as acidic catalyst. The chemicals were of reagent grade and used without further purification. All chemicals were purchased from Merck and Sigma Aldrich (Malaysia).

Dissolution of Condensed Tannins with Water-glycerol

The dissolution of tannin was done as per Cardona and Sultan (2015). The condensed tannin (Bondtite-345 from Bondtite Adhesives (Pty) Limited, Pietermaritzburg, South Africa) was a brown powder with a moisture content of approximately 10 to 12% by weight and 4 to 6% ash content. The approximate particles size of tannin powder used was between 10 µm to 50 µm. Prior to mixing with the liquid phenolic resins, the tannin was dissolved in water and glycerol mixture in the presence of sulphuric acid (1 % by weight). The solution mixture was prepared by adding 20 g of glycerol, 80 g of water, and 1 g of sulphuric acid and were heated from 30 °C to 80 °C. When the temperature reached 80 °C, about 70 g of tannin powder was slowly mixed into the solution until the powder dissolved. The mixture was stirred for 40 min at 80 °C.

The set parameters, namely mass of glycerol, mass of tannin powder, temperature, and stirring time were selected based on research conducted by Samil *et al.* (2005) and Cardona and Sultan (2015). Samil *et al.* (2005) investigated the dissolution of tannin in phenol, while Cardona and Sultan (2015) tested the dissolution of tannin in glycerol and used it for resin applications. The preliminary screening parameters are shown in Table 1. Changes were done on a few parameters based on the authors' early screening. To illustrate, the observations showed that all the 50 g of tannin added into the solvent were dissolved in a very short period. Therefore, the initial point was reset at 60 g because the response for the experiment was based on undissolved value. The upper level was maintained at 100 g, as shown in Table 1. For the mass of glycerol used, the preliminary parameter was as shown in Table 1. The solvent was prepared by mixing 10 g of glycerol with 90 g of water to form 100 g of glycerol-water solvent. Based on the preliminary results, for 100 g of tannin powder, dissolutions were observed to partially happen at a very specific amount of glycerol used, *i.e*. in the range of 10 to 30 g of glycerol. Control experiments were also

performed for both 100 g glycerol and 100 g water sample. Therefore, the set level for mass of glycerol used was from 10 to 30 g. Then, for the synthesis temperature, the set range was from 50 to 100 °C. From the preliminary screening, synthesis temperature that is higher than 60 °C will easily dissolve the tannin powder. The new synthesis temperature was set between 60 and 100 °C. To partially dissolve 100 g of tannin in glycerol-water solvent (10:90), the required stirring time was 20 min. It took about 60 min to fully dissolve the tannin powder. If the stirring time was prolonged for more than 60 min, then the glycerol and water mixture was evaporated sticky tannin coagulant was formed. Thus, the level for stirring time was set between 20 to 60 min.

Table 1. Set Data for Pre-treatment (One Factor at A Time (OFAT))

Experimental Parameters	Preliminary Screening Ranges		Ranges		Intervals	References
Mass of Tannin Powder (g)	50	100	60	100	10	(Wolfson *et al.* 2007; Cardona & Sultan, 2015)
Mass of Glycerol (g)	0	100	10	30	5	
Temperature (°C)	50	100	60	100	10	
Stirring Time (minutes)	20	60	20	60	10	
Total Number of Experimental Sets for OFAT				60		
* Water was added to glycerol to produce 100 g of solvent						

The screening of the dissolution tannin powder was further studied using a one factor at one time (OFAT) approach, as done by Campolongo *et al.* (2007). The effects of parameters such as mass of tannin powder, mass of glycerol used, temperature and stirring time were analyzed as per data presented in Table 1. The best value of each parameter was determined by evaluating the percentage of undissolved tannin while the other parameters were kept constant. The amount of undissolved tannin is the key parameter that was measured in this work.

Viscosity Assessment

A simple viscosity assessment was done on the dissolved tannin to measure the viscosity of the solution. The dynamic viscosity of the dissolved tannin was measured using Brookfield Viscometer (DV2T) using cylindrical spindle LV-04(64) at 200 RPM.

Evaluation of Undissolved Tannin Percentage

The tannin mixture was further diluted in water with the ratio of tannin mixture to water set at 1:4. The solution was centrifuged at 10000 RPM for 10 min to separate the micro particles in the undissolved tannin, as suggested by Shah et al. (2009). The remaining solid was weighed. The procedures were repeated for all samples.

Optimizing Tannin Dissolution with Response Surface Methodology (RSM)

The mass of tannin powder, mass of glycerol, temperature, and stirring time were optimized to obtain the lowest amount of undissolved tannin. Response surface methodology (RSM) using Design-Expert (version 7.0.0, Statease, Minneapolis, USA) software as the tool was used to optimize the experimental data. A factorial central composite design (CCD) was employed for the four parameters with six replications at the center point as suggested by Design-Expert software version 7.0.0 (Statease, Minneapolis, USA). The critical F-value lack of fit value was less than 5.05, *i.e.*, the F-value lack of fit for the dissolution of tannin model was 2.21. Tanyildizi *et al.* (2005), Mahalik *et al.* (2010),

and Behera *et al.* (2018) claimed there are three major steps involved in optimization using RSM. These steps covered statistically designed experiments, estimation of coefficients in a mathematical model, prediction of response, and checking the adequacy of the model within the experimental setup. The method was applied to assess the effects of operating parameters on the percentage of undissolved tannin after the dissolution process. A polynomial equation was fitted to the data to obtain the best fit model. The statistical significance was examined based on the analysis of variance (ANOVA) results and the response surface plots were generated using the same software.

The factors were selected based on the results of the OFAT test. The interactions were further evaluated using the RSM. Four independent variables were chosen as follows: mass of glycerol (X_1, g), mass of tannin powder (X_2, g), temperature (X_3, ^oC), and stirring time (X_4, min). The range and level of the factors varied according to the experimental design. These four variables together with their respective ranges were found to be critical parameters for the percentage of undissolved tannin. The set coding for the RSM approach was as shown in Table 2. From four independent variables, thirty experimental runs were designed and carried out for the dissolution process. The aim of the experimental design was to optimize the response variables (Y). A suitable approximation for the true correlation between independent variables and response surfaces are needed to be find (Gunaraj and Murugan 1999; Behera *et al.* 2018). The experimental run was randomized in order to reduce the error and effect of the independent variables. Experimental data were fitted to a second-order polynomial model and regression coefficients can be obtained (Baş and Boyacı 2007). The generalized second-order polynomial model, Eq. 1 was used to evaluate this effect of parameters on the response,

$$Y = \beta_0 + \sum^4_{i=1} \beta_i X_i + \sum^4_{i=1} \beta_{ii} X^2_i + \sum^3_{i=1} \sum^4_{j=i+1} \beta_{ij} X_i X_i \quad (1)$$

where Y represents the response of undissolved tannin, β_0 is a constant, β_i, β_{ii}, and β_{ij} are the constant coefficients, and x_i and x_j are the coded independent variables.

Table 2. Coding of Experimental Parameters and Related Levels

Experimental Parameters	Units	Symbols	Coded Values			Response*
			Low (-1)	Medium (0)	High (+1)	
Mass of Glycerol	g	x_1	10	15	20	Percentage of undissolved tannin
Mass of Tannin Powder	g	x_2	55	65	75	
Temperature	^oC	x_3	75	80	85	
Stirring Time	minutes	x_4	35	40	45	
*The response was keyed in base on the individual calculation						

RESULTS AND DISCUSSION

Effect of Mass of Tannin Powder, Mass of Glycerol, Temperature and Stirring Time

The effect of each operating parameter was evaluated. The main objective was to have the highest amount of tannin dissolved in the solution by measuring the amount of undissolved tannin in each set of the experiment. Since the amount of tannin used in the experiment differed due to the difference dissolution capacity of the prepared solution, therefore all results were presented in percentage (mass of undissolved tannin/mass of

tannin powder) form. Inclusion of tannin in the laminate resin will improve the flexural properties of the composite and reduce its brittleness. Furthermore, the laminate resin coats and protects the surface of the material. Table 3 represent the results from one factor at a time (OFAT) screening.

Table 3. Representative Results from OFAT Screening for the Lowest Percentage of Undissolved Tannin

Set of Experiment	Experimental Parameters				Percentage of Undissolved Tannin (%)	Mass of Undissolved Tannin (g)
	Mass of Glycerol (g)	Mass of Tannin Powder (g)	Temperature (°C)	Stirring Time (min)		
1	15	75	70	30	12.75	9.57
2	20	65	70	30	11.96	7.77
3	20	75	80	40	11.19	8.39
4	20	75	80	40	11.35	8.51

Optimized conditions were found when each parameter was varied at a time. The effect of mass of glycerol, tannin powder, temperature, and stirring time helps in minimizing the use of raw materials in dissolution procedure while maximizing the tannin powder to be dissolved. The data presented in Table 3 are the representative data of all OFAT sets of experiments. For the given experimental parameters, the presented undissolved percentage represents the best condition to dissolve tannin. Table 3 presents the results of four best experiments from the 100 sets performed for OFAT experiments. The result shows that the lowest percentage of undissolved tannin ranged between 11% and 13% by weight. The presented values obtained from the OFAT experiments were used as the midpoint in the RSM coded values shown in Table 2.

Fully dissolved tannin resulted in a smooth and homogenous solution, as illustrated in Fig. 1 (a). It was noted that adding tannin powder in large amounts at one time led to the formation of solid tannin coagulate, while addition of an excessive amount of tannin powder led to the dispersion of the tannin powder in the solution as shown in Fig. 1 (b). Adamczyk *et al.* (2017) highlighted the interactions of tannin with other compounds for example protein, various organic nitrogen compounds as well as enzyme that might also produce complex in the forms of coagulate. However, in this study, no such compounds were mixed other than water and glycerol as the solvent. Therefore, formation of other complex compounds in the form of coagulates is assumed not to be present.

(a) **(b)**

Fig. 1. Dissolved tannin solution (a) Almost fully dissolved tannin solution (b) Dispersion of tannin

Dissolved tannins in liquid form are more reactive compared to solid tannins (Samil *et al.* 2005; Kotz *et al.* 2009). Active sites available at the cyclic ring in catechin, as shown in Figs. 2 and 3, will provide places to other compound to form hydrogen bonding and to make a much stronger bond (Pizzi and Scharfetter 1978; Jahanshaei *et al.* 2012b). The addition of solid tannin, on the other hand, will only act as filler, *i.e.* physical bonding, and did not initiate any chemical bonding which is much stronger (Cardona and Sultan 2015).

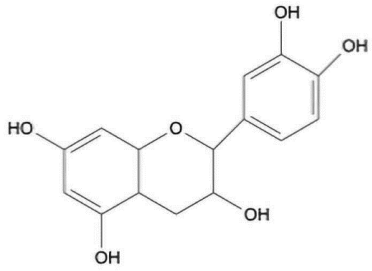

Fig. 2. Chemical structure of catechin (Wolfson *et al.* 2007)

The possible reaction that occurred between catechin from the dissolved condensed tannin components with glycerol and water is as illustrated in Figs. 3 and 4.

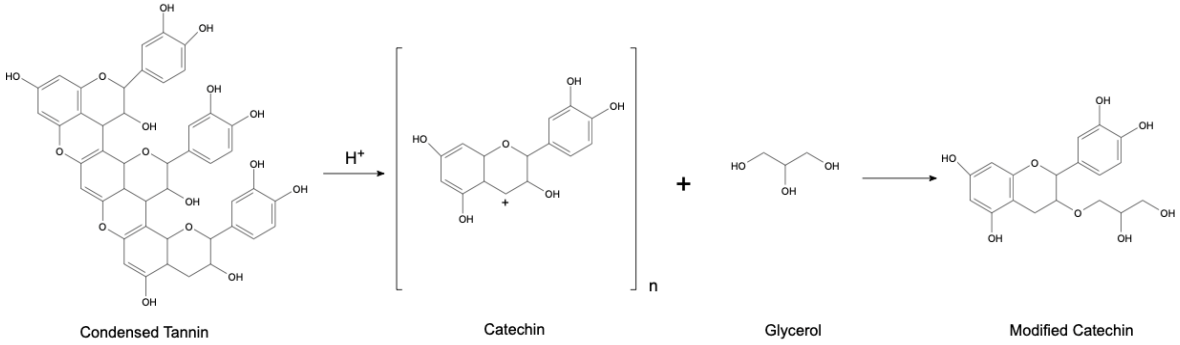

Fig. 3. Possible reaction between catechin and glycerol in the presence of sulfuric acid (Samil *et al.* 2005; Cardona and Sultan 2015)

Figures 2 and 3 show the possible reaction between catechin molecule with glycerol as well as with water. Addition of water to glycerol reduced the viscosity of the water-glycerol solution. The water molecules interact with glycerol and catechin at the OH sides through hydrogen bonding (Cuevas-Valenzuela *et al.* 2014; Botten *et al.* 2015).

Viscosity of Dissolved Tannin

Viscosity of dissolved tannin in a different amount of glycerol was compared. Cardona and Sultan (2015) used 100% glycerol as the solvent and had noted the high viscosity of the solution when tannin was dissolved in it. However, there is no specific viscosity value noted by them. According to them, this highly viscous solution led to the difficulty in handling the solution during the downstream processing. Based on the recommendations by Gillern *et al*. (1978) and Georgia Pacific Chemicals (2020), the suitable working viscosity of the final laminate resin is within the range 400 to 600 cP. Therefore, this study explored a possible method to reduce the viscosity of dissolved tannin and glycerol mixture by adding a co-solvent, *i.e.* water. The viscosity value of the produced solution shall be smaller than the described value.

Table 4. Viscosities of Dissolved Tannin in Water-glycerol Acid Solution

Mole Fraction of Glycerol used to dissolve 75 g of Tannin Powder	Viscosity (cP)
0.02	350
0.05	383
0.08	417

Table 4 presents the viscosity of dissolved tannin solution recorded for samples prepared using 75 g of tannin dissolved in 100 g of solution with 10 to 30% glycerol content (0.02 to 0.08 mole fraction of glycerol). It can be noted that all samples exhibited viscosity values lower than 500 cP, and as the amount of glycerol was increased by 20%, the viscosity increased by 60 cP. It can be deduced that if the amount of glycerol is further increased, *i.e* to 100%, the extrapolated viscosity value of the prepared solution will be approximately 650 cP. It was found from this experiment that water alone has the capability of dissolving tannin up to 30% by weight. This was also supported by other work (Hussein *et al.* 2011). Taking this into account, it shows that a mixture of water and glycerol will be able to improve the dissolution of tannin by up to 12% more while keeping the final viscosity of the solution within 400 ± 50 cP.

Even though the addition of highly viscous dissolved tannin into resin will improve its chemical bonding (Cardona and Sultan 2015), the downstream processing will encounter process challenges. In particular, unpliable resins can lead to uneven coating, Addition of water in reducing the viscosity also created problems. For example, the high content of water will lead to the formation of voids within the composite structure due to the fast evaporation of water. These voids create defects on the composite (Bajia *et al.* 2007; Pilato 2010; Cardona and Sultan 2015). Laminate resins with high water content will also require a prolonged post-curing period, which will affect the production time. Therefore, the proper ratio of glycerol and water had to be identified to mitigate both of these processing problems. In this study, viscosity values were only used as a marker in OFAT study and the undissolved amount tannin was used as response in the RSM.

Optimization of Parameters

From the OFAT screening, the new conditions for center point in RSM were set as tabulated in Table 5.

Table 5. New Set Data for Dissolution

Experimental Parameters	Units	Symbols	Set Point
Mass of Glycerol	g	x_1	15
Mass of Tannin Powder	g	x_2	65
Temperature	°C	x_3	80
Stirring Time	min	x_4	40

The RSM was used to evaluate the effects of these independent parameters on the response percentage of undissolved tannin and to create a model between these parameters. A second-order multi regression model was constructed as a function of the mass of glycerol (X_1), mass of tannin powder (X_2), temperature (X_3), and stirring time (X_4) on the predicted response percentage of undissolved tannin (Y). The statistical and regression coefficient significance of the model was checked against the probability (p-value). A P-value of less than 0.05 was applied to validate the significance of the model and each of the parameters.

The percentage of undissolved tannin (*Y*) model for the dissolution of tannin is illustrated using second order polynomial equation as follows:

$$Y = 10.20 + 0.43 X_1 + 0.26 X_2 + 0.27 X_3 + 0.085 X_4 - 0.12 X_1 X_2 - 0.40 X_1 X_3 - 0.19 X_1 X_4 - 0.030 X_2 X_3 - 0.031 X_2 X_4 + 0.42 X_3 X_4 \quad (2)$$

To assess the importance of each parameter, the effects of single and interactive two parameters were evaluated. Goodness of fit of models was analyzed based on the regressed values between the model and the individual coefficients, as proposed by Lucas (2010) and Behera *et al.* (2018). Table 6 presents the ANOVA values for the data generated by Eq. 2, which reflects the amount of undissolved tannin in the process. Parameters that have F values which are larger than 3.30 and P values that are less than or equal to 0.0005 significantly affected the dissolution process, *e.g.* mass of glycerol, mass of tannin powder, and dissolution temperature. The effect of stirring time was found to be negligible. Figure 4 depicts the comparison between the actual and predicted for the percentage of the undissolved tannin.

The low p ($p \leq 0.0001$ and less than 0.0005) and the high F values ($F = 192.2$) indicated that the model developed was highly significant. The R^2 value between the actual data and the model was calculated to be 0.9945. Analysis of the R^2_{adj} shows that the value was 0.9893, indicating that the R^2 estimator was not biased.

The P-values for 'Lack of fit' were found to be greater than 0.05 and were insignificant. This shows that predicted values from the developed model fit to the actual experimental response data. About 19.76 % of the lack of fit for p-value was attributed to noise, *i.e.* parameters that were not accounted for in the experiment, *e.g.* change in surrounding temperature, amount of acid catalyst used, *etc*. Low value of the coefficient of variation (CV) which is 1.06 %, indicates the preciseness and repeatability of the response collected from the experiment (Mohajeri *et al.* 2010).

Figure 5 compares the distribution of the residual with that of a normal distribution. It shows that the residual fit in to the normal distribution profile with some deviation at points (x_1= 25 g, x_2= 65 g, x_3= 80 g, x_4= 40 g with *Y*=14.68 % and x_1= 5 g, x_2= 65 g, x_3= 80 g, x_4= 40 g with Y= 13.28 %).

ANOVA results tabulated in Table 6 were carefully studied. It was found that all variables had a significant effect on the response. The combined effect between mass of glycerol (x_1) and mass of tannin powder (x_2), between mass of glycerol (x_1) and temperature (x_3), between mass of glycerol (x_1) and stirring time (x_4) and between temperature (x_3) and stirring time (x_4) were also significant relative to the response. Individual parameters, such as mass of glycerol (x_1), mass of tannin powder (x_2), and temperature (x_3) imposed greater effect compared to that of stirring time (x_4). The quadratic effect of all variables which are mass of glycerol (x_1), mass of tannin powder (x_2), temperature (x_3), and stirring time (x_4) was very significant and had the highest effect on the amount of undissolved tannin.

Table 6. ANOVA Table (partial sum of squares) for Quadratic Model (response: percentage undissolved tannin)

Source	SS	DF	MS	F-value	P-value	Remarks
Model	39.22	12	3.27	192.19	<0.0001	Significant
X_1 (Mass of Glycerol)	4.39	1	4.39	301.30	<0.0001	Significant
X_2 (Mass of Tannin Powder)	1.57	1	1.57	107.87	<0.0001	Significant
X_3 (Temperature)	1.77	1	1.77	121.72	<0.0001	Significant
X_4 (Stirring Time)	0.17	1	0.17	11.86	0.0036	Significant
$X_1 X_2$	0.23	1	0.23	15.60	0.0013	Significant
$X_1 X_3$	2.62	1	2.62	179.67	<0.0001	Significant
$X_1 X_4$	0.57	1	0.57	39.24	<0.0001	Significant
$X_2 X_3$	0.014	1	0.014	0.98	0.3378	
$X_2 X_4$	0.015	1	0.015	1.04	0.3235	
$X_3 X_4$	2.76	1	2.76	189.38	<0.0001	Significant
X_1^2	24.86	1	24.86	1705.42	<0.0001	Significant
X_2^2	0.29	1	0.29	20.11	0.0004	Significant
X_3^2	1.15	1	1.15	78.91	<0.0001	Significant
X_4^2	0.95	1	0.95	64.94	<0.0001	Significant
Residual	0.22	15	0.015			
Lack of Fit	0.18	10	0.018	2.21	0.1976	Not Significant
Pure Error	0.04	5	0.0081			
Correlation Total	39.43	29				
R^2						0.9945
Adj R^2						0.9893
Coefficient Variation						1.06%
SS- sum of squares; DF- degrees of freedom; MS- mean square						

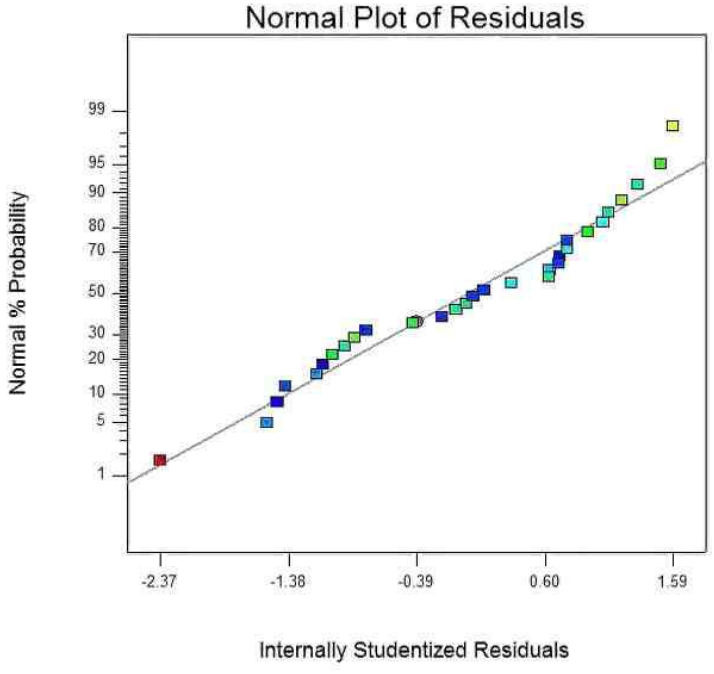

Fig. 4. The studentized residuals and normal percentage probability plot of percentage of undissolved tannin from tannin dissolution process

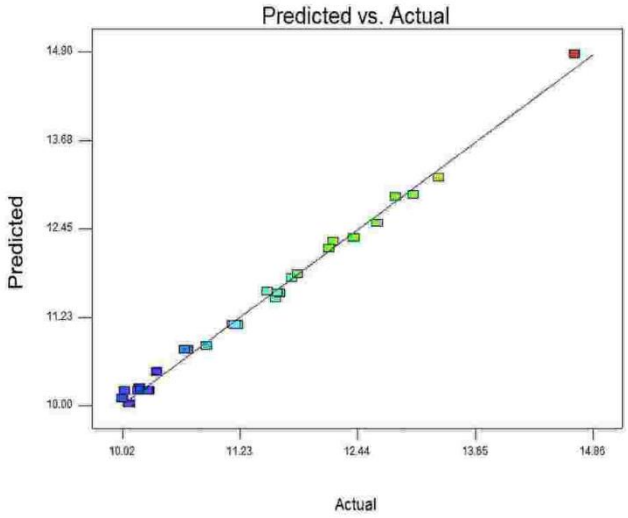

Fig. 5. Comparison of the actual and predicted value of the percentage of undissolved tannin

In order to study the individual and interaction effect of four variables including mass of glycerol, mass of tannin powder, temperature, and stirring time of tannin dissolution for minimizing the percentage of undissolved tannin produced, the response surface methodology was used and three-dimensional surface plots including two-dimensional plots were obtained and are shown in Fig. 6.

Figs. 6 (a) and (b) show the interaction between mass of glycerol (x_1) and mass of tannin powder (x_2) in tannin dissolution process. When the mass of glycerol was increased in the range up to 15 g and the mass of tannin powder was 65 g, a gradual reduction in the response was recorded. The percentage of undissolved tannin is 10.02 % by weight, where 6.51 g of undissolved tannin produced, while 58.49 g of tannin powder was dissolved. Increased of mass of glycerol increased the capability of water-glycerol solvent to dissolve tannin powder. Increasing the amount of glycerol used as solvent will indirectly more sites for binding with tannin, *i.e.* more tannin will be dissolved in the glycerol-water solvent (Bogardus 1984; Wolfson *et al.* 2007).

The interaction between mass of glycerol (x_1) and temperature (x_3) is shown in Figs. 6 (c) and (d). When the temperature (x_3) was increased from 80.00 to 85.00 °C, the percentage of undissolved tannin decreased gradually by almost 5% by weight. Here, the percentage of undissolved tannin was about 10.18 % by weight. Out of 65.00 g of tannin powder added into the solution, approximately 6.62 g tannin was not dissolved. When the temperature was further increased, *i.e.* higher than 80.0 °C, the percentage of undissolved tannin was gradually increased. This observation indicated that the operating temperature plays a key role in the dissolution process. Fast evaporation of the water-glycerol solution at a temperature much higher than 80.0 °C might occur because the boiling point of the mixture is at 101.0 °C. Due to this, there was a reduced amount of water-glycerol solution available to be mixed with the tannin solution leading to the poor dissolution of tannin.

The effect of stirring time on the mixing process is shown in Figs. 6 (e) and 6 (f). Here, the interaction between mass of glycerol (x_1) and stirring time (x_4) was evaluated with a mass of glycerol for the dissolution process set between 10 and 20 g, and the stirring time set between 35 and 45 min. The lowest percentage of undissolved tannin was 10.29% by weight when the mass of glycerol used was 14 g and the stirring time was set at 40 min.

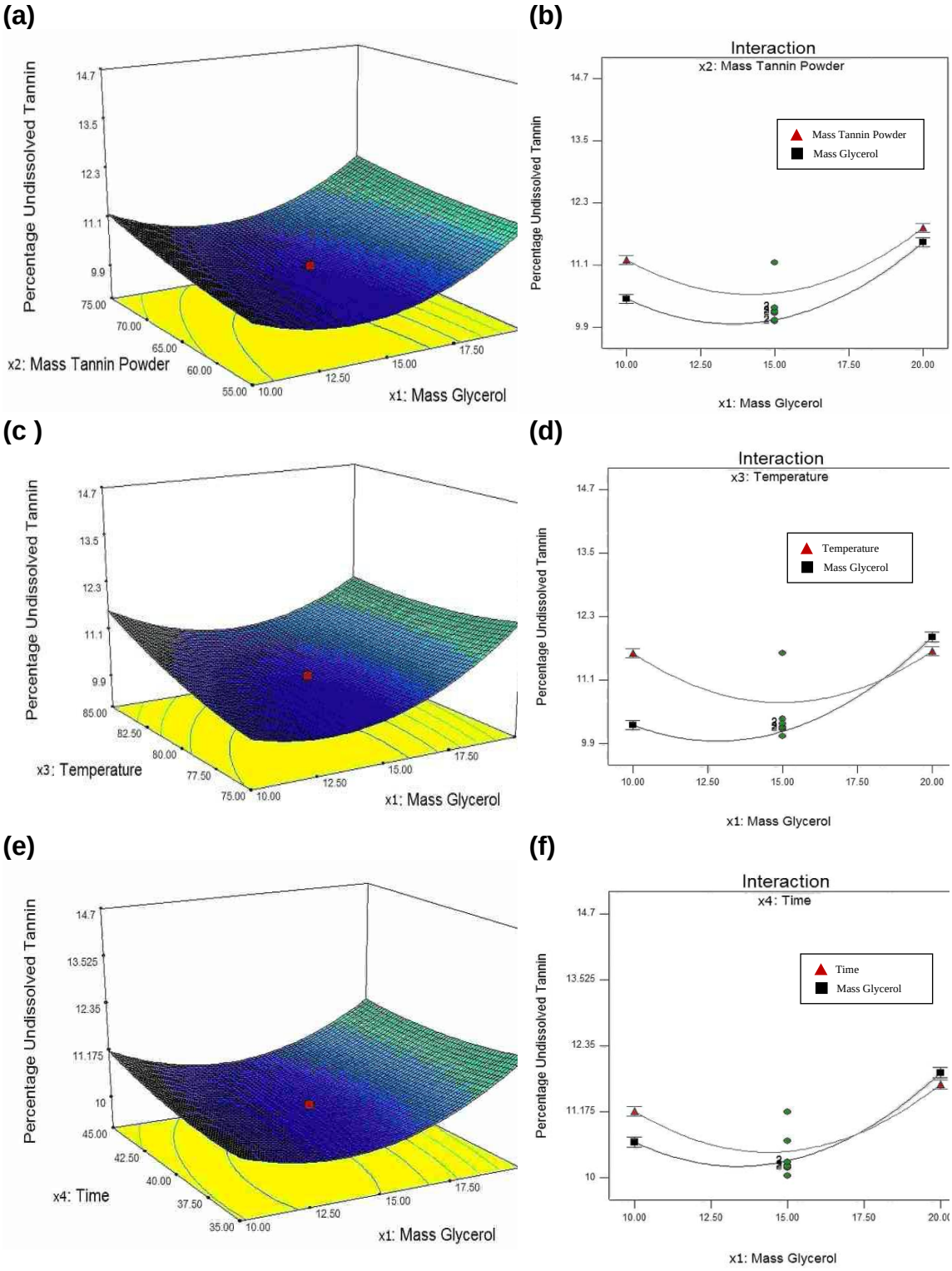

Fig. 6. Response surface curves of (A) Three-dimensional surface plot and (B) interaction mass of glycerol (x_1) *vs.* mass of tannin powder (x_2) for the percentage of undissolved tannin (%) produced. (C) Three-dimensional surface plot and (D) interaction mass of glycerol (x_1) *vs.* temperature (x_3) for the percentage of undissolved tannin. (E) Three-dimensional surface plot (F) interaction mass of glycerol (x_1) *vs.* stirring time (x_4) for the percentage of undissolved tannin

At a much shorter stirring time, *i.e.* 35 min, the undissolved percentage was increased to 10.43% by weight. These findings show that the percentage of undissolved tannin was reduced as the time for stirring was prolonged to 40 min. Mixing enhances the diffusion of tannin from the bulk to the solution (Kondili *et al.* 1993). Stirring also improves both the heat and mass transfer rates of the process and reduced the mass transfer resistance (Wang and Qin, 2007; Gabelle *et al.* 2012). The RSM software also proposed the best parameters set to dissolve tannin for this experiment as shown in Table 7. The predicted percentage of undissolved tannin was 10.27%.

Table 7. Proposed Set Data for Dissolution of Tannin by RSM Software

Experimental Parameters	Units	Symbols	Data
Mass of Glycerol	g	x_1	13.56
Mass of Tannin Powder	g	x_2	75
Temperature	°C	x_3	75
Stirring Time	minutes	x_4	44.13

A verification experiment was conducted at similar conditions. Ten parallel experiments were carried out. The standard deviation of the ten verification experiments was ± 0.07. The difference between the predicted and the actual values was about 0.43%, only indicating the adequacy of the model prediction approach (Yi *et al.* 2010; Sulaiman *et al.* 2014; Pagliaro 2017). The value was also within the acceptable range because it was much smaller than the set threshold of 5%, as suggested by Design-Expert software version 7.0.0 (Statease, Minneapolis, USA). The small percentage almost 0 % indicates that the settings seem to achieve favorable results for all responses as a whole. The value of undissolved tannin obtained from the optimized conditions was compared to that obtained from similar set conditions performed during the preliminary experiment. Table 8 shows the comparison of the percentage undissolved tannin.

Table 8. Comparison of Percentage Undissolved Tannin with Different Screening Stage and Optimize Condition

Screening Conditions				
Experimental Parameters	Units	Data	Average Percentage of Undissolved Tannin (%)	Mass of Undissolved Tannin (g)
Mass of Glycerol	g	20.00	14.72	11.04
Mass of Tannin Powder	g	75.00		
Temperature	°C	80.0		
Stirring Time	minutes	40.00		
Optimize Conditions				
Experimental Parameters	Units	Data	Average Percentage of Undissolved Tannin (%)	Mass of Undissolved Tannin (g)
Mass of Glycerol	g	13.56	10.32	7.74
Mass of Tannin Powder	g	75.00		
Temperature	°C	75.0		
Stirring Time	minutes	44.13		

CONCLUSIONS

The focus of this study was to investigate the dissolution of tannin powder in water-glycerol acid solution to produce a less viscous tannin solution and to optimize the tannin powder dissolution process. From this study, the following conclusion can be made:

1. The optimization approach improved the dissolution process. After optimization, the dissolution of tannin powder in water-glycerol acid solution was improved from 86% to 89.68% (by weight), with the optimum dissolution condition of mass of glycerol (13.56 g), mass of tannin powder (75 g), temperature (75 °C), and stirring time (44.13 minutes).
2. Mass of tannin powder and glycerol and the temperature of reaction significantly affect the dissolution of tannin.
3. The deviation of undissolved tannin value predicted by the model proposed by RSM and that obtained from the verification experiment was within the acceptable range (5%).
4. It is possible to reduce the amount of undissolved tannin powder in dilute glycerol. With addition of water and dilute glycerol the mixing of the solution will become much easier and thus produce less viscous dissolved tannin solution.

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