Journal of Engineering Science and Technology
Special Issue on SOMCHE 2014 & RSCE 2014 Conference, January (2015) 22 - 31

SYNTHESIS OF GREEN ZERO-VALENT IRON USING POLYPHENOLS FROM DRIED GREEN TEA EXTRACT

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Abstract

Green zero-valent iron (ZVI) was synthesised using polyphenols from green tea extract as reducing agent and ferric chloride ($FeCl_3$) as iron precursor. Polyphenols were extracted through microwave-assisted extraction from dried green tea leaves with varying green tea-to-solvent ratio (1:18-1:22 g/mL). During ZVI synthesis various green tea extract flow rate (8-17 mL/min) and ferric chloride-to-green tea extract ratio (1:1-1:3 mL/mL) were investigated. The most favourable conditions for the synthesis of green ZVI was determined based on the yield, rate of oxidation and surface area of the metal product. The conditions which gave the highest yield of 86.48% were the following: green tea-to-solvent ratio of 1:20, green tea extract flow rate of 8mL/min and a $FeCl_3$-to-green tea extract ratio of 1:2. On the other hand, the conditions which gave the smallest particle size (8.82 nm) were the following: green tea-to-solvent ratio of 1:20, a green tea extract flow rate of 17 mL/min and a $FeCl_3$-to-green tea extract ratio of 1:3. It was determined that the green tea extract flow rate was the only factor that significantly affected both the particle size and the yield. The factors tested did not have significant effect on the rate of oxidation of the metal product.

Keywords: Green zero valent iron, Iron precursor, Green tea extract, Polyphenols, Nano particles.

1. Introduction

The use of zero-valent iron (ZVI) is becoming attractive in the treatment of various organic and inorganic pollutants because of its unique properties of being able to transform aquatic pollutants into less benign products. Although ZVI is

commercially available, its synthesis in laboratory scale has been the subject of research activities with the aim of modifying its properties focusing on particle size. A smaller particle size means higher surface area per unit mass making the material more reactive [1]. Thus, nano-sized ZVI (NZVI) has become popular in remediation technology. NZVI particles are typically 5-40 nm sized Fe^0/Fe-oxide particles [2].

Common compounds used in the synthesis of ZVI are sodium borohydride ($NaBH_4$) as reducing agent and ferric chloride ($FeCl_3$) as iron precursor. Sodium borohydride has been proven to be effective in producing NZVI, but it is hazardous and expensive. In addition, the synthesised ZVI easily corrode during storage making it less potent in degrading pollutants. Commercial ZVI are usually coated which require pre-treatment before use.

Recently, several researches have demonstrated that the use of plant extracts as reducing agent has the potential to produce stable dispersion of iron nanoparticles [3] producing what is called "green ZVI" The use of plant extracts is environmentally friendly, producing non-toxic products. The plant extracts are rich in polyphenols, which have high antioxidant property. Some examples of plants with high phenolic content that have previously been used to synthesise ZVI, include oak, pomegranate, green tea, mulberry, eucalyptus and black tea [4]. In the current study, green tea leaves were used. Generally, the polyphenols in green tea leaves comprise about 30% of the dry leaf weight [5]. The most abundant of the polyphenols are catechins, other groups present are flavanols, flavanones, phenolic acids and glycosides [6]. These polyphenols are soluble in various solvents such as water, ethanol, methanol and acetone.

The formation of ZVI using polyphenols occurs through the binding of a catecholate or gallate ligand to Fe^{3+} forming semiquinone which reduces Fe^{3+} to Fe^{2+} with the formation of quinone species [7]. Though there is no definite explanation for the formation of Fe^0 (ZVI), there are speculations that this is due to the reduction potential of catechin which is sufficient to reduce Fe^{2+} to Fe^0 [8]. Mystrioti et al. [9] suggested the overall mechanism for the formation of Fe^0 using polyphenols and is shown in Eq. (1); where *Ar* represents the aromatic ring and *n* is the number of groups oxidised by Fe^{3+}.

$$nFe^{3+} + 3\left(Ar - OH\right)_n \rightarrow nFe^0 + 3n\left(Ar = O\right) + 3nH^+ \quad (1)$$

The use of plant extracts as substitute to sodium borohydride as reducing agent in the synthesis of ZVI is a relatively new technology and little information on the subject is available in literature. Previous studies on green ZVI show that the plant extracts and the iron precursor were simply mixed and the product characterized. In the synthesis of ZVI using sodium borohydride, factors such as reducing agent delivery rate to the iron precursor as well as the ratio of the reducing agent to the iron precursor have been investigated as these factors were found to significantly affect the characteristics and reactivity of the ZVI. The effects of these factors are not known when plant extracts are used.

2. Materials and Methods

2.1. Materials

The main raw materials used in the study were dried green tea leaves acquired from Ten Fu's Tea (Beijing, China). The leaves came from a single batch of

harvest as certified by the supplier to ensure uniform product and eliminate factors which could potentially affect the results of this study. Aqueous ethanol solution was used as the solvent due to its relatively high dielectric constant making it suitable for microwave absorption [4] and it also served as additional protective layer for the ZVI, preventing it from oxidation. Folin-Ciocalteu reagent and sodium carbonate solution were used for the measurement of the total phenolic content of the extract. Analytical grade ferric chloride was used as precursor of iron particles.

2.2. Extraction of polyphenols

The green tea leaves were ground using mortar and pestle and were sieved using mesh 20 Tyler screen standard. All particles that passed through mesh 20 (under 0.841 mm) were collected and stored in amber bottles prior to use.

Varying amounts of green tea (4.55, 5 and 5.55 g) were weighed and dissolved in 100 mL of 50% (v/v) aqueous ethanol solution based on the study of Pan et al. [6]. The resulting solution was placed in a 500-mL cylindrical flask and shaken for 5 minutes. The flask was then placed inside the microwave oven by fastening it to the bottom of the condenser attached on top of the oven. The extraction condition was set at 85ºC, 700 W microwave power and extraction time of 4 minutes. A cooling time of 5 minutes was allotted before removing the flask. The contents were then filtered with the use of Buckner funnel. The filtrate was placed in amber bottles while solid residue was discarded.

2.3. Synthesis of ZVI

2.3.1. Experimental design

Preliminary runs were conducted to determine a set of appropriate values for each factor considered in the main experimental runs. The set of appropriate levels of factors used in the main experimental runs is shown in Table 1. Experiments were conducted following Taguchi method with $L_9 3^3$ orthogonal array.

Table 1. Factor-level combination used in the main experimental run.

Levels	Green tea-to-solvent ratio (*w/v*)	Green tea extract delivery rate (*mL/min*)	$FeCl_3$- to-green tea extract ratio (*v/v*)
1	1:18	8.0	1:1
2	1:20	12.5	1:2
3	1:22	17.0	1:3

The concentration of the $FeCl_3$ solution was fixed at 0.1M. The ratio of ferric chloride to extract was varied by varying the volume of green tea extract while keeping the volume of ferric chloride solution constant. The responses are yield, rate of oxidation and particle size of the metal product.

The yield of green ZVI is defined as the percentage by weight of ferric ion in the original ferric chloride solution that was converted to ZVI and recovered from the synthesis solution. This was calculated using Eq. (2).

$$yield = \frac{\text{weight of } Fe^0 \text{ produced and recovered from the synthesis solution}}{\text{weight of } Fe^{3+} \text{ in the original } FeCl_3 \text{ solution}} \quad (2)$$

The rate of oxidation of ZVI was determined qualitatively through daily visual observation of its colour for a period of 12 days. The faster the transition of colour of the synthesised ZVI from black to brown, the greater is its rate of oxidation.

2.3.2. Preparation and synthesis procedure

A 0.1 M solution of ferric chloride was prepared by dissolving a fixed amount (2.703 g) of ferric chloride ($FeCl_3 \cdot 6H_2O$) in 100 mL of distilled water. The water used was pre-cooled to a temperature of 2-5 ºC for 30 minutes as suggested by Pan et al. [6] to decrease the amount of dissolved O_2 which might consume the reducing agent. The synthesis process of green ZVI was based on the method of Hoag et al. [3].

The ferric chloride solution was stirred for five minutes and then placed in a triple-neck flask. Varying amounts (100 mL, 200 mL and 300 mL) of green tea extract was placed in a burette which was added to the ferric chloride solution by drop-wise addition to control the flow rate of the reducing agent. Varying flow rates of the reducing agent were used in the experiment (8.0, 12.5 and 17.0 mL/min). While the green tea extract was added, the contents of the reactor were continuously stirred using a mechanical stirrer attached to the top of the flask. When all of the extract has been added, the stirring process was continued for 10 minutes. The experimental set-up for the synthesis of ZVI is shown in Fig. 1.

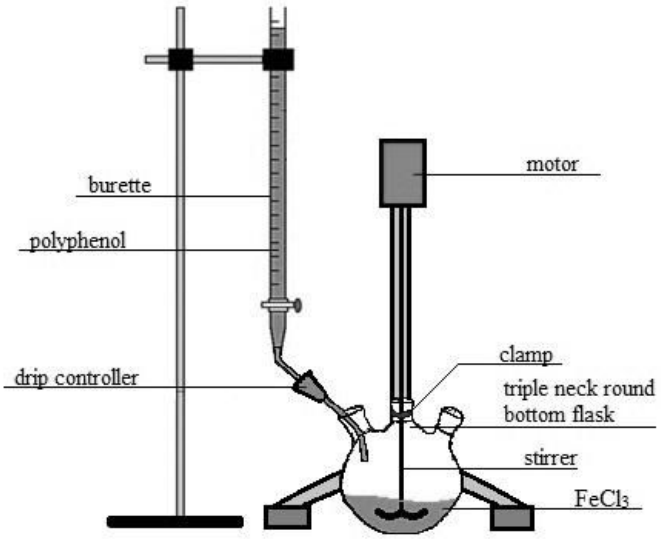

Fig. 1. Schematic diagram of synthesis set-up.

The resulting solution was centrifuged for 30 minutes at 5000 rpm. The precipitate generated was then gathered and placed into test tubes which were then placed in a freeze drier (LABCONCO) for 24 hours. After freeze drying, the

dried particles were stored inside sealed vials which were placed inside zip-locked plastic bag and kept in desiccator before analysis.

Confirmatory runs were conducted using the conditions that gave the highest yield and lowest particle size.

2.4. Analytical techniques

2.4.1. Determination of polyphenol content of extract

The polyphenol content was determined through Folin-Ciocalteu assay patterned after Naidu et al. [10] and expressed as gallic acid equivalent per liter (mg GAE/L). The solvent from the green tea extract solution was first removed via rotary evaporator set at 80ºC for 15 minutes. The solvent-free extract was treated with Folin-Ciocalteu reagent and 7% sodium carbonate solution. Gallic acid served as the reference.

Standard solutions of gallic acid with different concentrations (100, 300, 500, 700 and 900 mg/L) were prepared and used to generate the calibration curve. A sample of 0.4 mL of the gallic acid solution was placed in a 10 mL volumetric flask containing 3.6 mL distilled water. To this mixture, 0.4 mL of Folin-Ciocalteu reagent was added and agitated for 5 minutes. Afterwards, 0.4 mL of 7% aqueous solution of sodium carbonate was mixed to each flask, and filled to the 10 mL mark with the addition of distilled water. A blank was also prepared by repeating the same steps but replacing the gallic acid solution with distilled water. After 90 minutes, the light absorbance for each sample was measured at 760 nm using a UV-Vis spectrophotometer (Hitachi, U-2900). A calibration curve was formed by plotting the gallic acid concentration (y-axis) versus the measured absorbance (x-axis). The green tea extracts were treated similarly and the total phenolic content was determined through the calibration curve.

2.4.2. Characterisation of green ZVI

The average size of the synthesised green ZVI particles was determined through images obtained from transmission electron microscope (TEM - Hitachi HT7700, 120 kV). The size of the ZVI particles was manually measured from TEM images. Using Minitab 14, a histogram of the particle size distribution was generated to determine the mean particle size for every sample. The elemental percentage composition of the green ZVI particles was determined using a scanning electron microscope (SEM - JEOL 5300) equipped with energy dispersive X-ray (EDX). The percentage of iron in the samples was used to determine the yield.

2.5. Treatment of data

The data obtained from the various experiments were analysed using Minitab. Analysis of variance (ANOVA) at 5% significance level was used to determine factors which have significant effect on the responses. This can be seen from the P-value corresponding to each factor. A p-value of 0.05 or less indicates that

changing the value of the factor will have significant effect on the response. Main effects plots were also constructed to show how responses are changed with change in the value of the factor.

3. Results and Discussion

A summary of the results of experiments conducted is shown in Table 2. The results shown are based on the $L_9 3^3$ orthogonal array of the Taguchi design of experiment. For the rate of oxidation, the number of days before the particles changed colour from black to brown was recorded.

The main effects plots for yield and particle size are shown in Figs. 2 and 3 to illustrate how the factors affect the yield and particle size.

Table 2. $L_9 3^3$ orthogonal array of the Taguchi design of experiment with the responses.

	Factors			Responses			
Run	**Green tea-to-solvent ratio (*w/v*)**	**Green tea extract delivery rate (*mL/min*)**	**$FeCl_3$ to green tea extract ratio (*v/v*)**	**% yield (*w/w*)**	**Rate of oxidation (days before particles changed colour)**	**Average particle size (*nm*)**	**Polyph-enol content of extract (*mg GAE/L*)**
1	1:18	8.0	1:1	63.24	No change	17.04	1150.50
2	1:18	12.5	1:2	57.38	No change	16.46	1150.50
3	1:18	17.0	1:3	49.38	No change	8.82	1150.50
4	1:20	8.0	1:2	86.48	4	16.86	1103.14
5	1:20	12.5	1:3	59.70	No change	11.07	1103.14
6	1:20	17.0	1:1	45.94	No change	9.70	1103.14
7	1:22	8.0	1:3	64.13	9	23.30	973.30
8	1:22	12.5	1:1	45.12	No change	21.61	973.30
9	1:22	17.0	1:2	43.75	No change	15.75	973.30

3.1. Effect of green tea-to-solvent ratio

It can be observed that as the amount of solvent used for extraction increased relative to green tea there was a decrease in the total phenolic content of the extract. Sufficient amount of solvent is needed for the material to be totally immersed, but excessive amount of solvent gives lower recovery because of non-uniform distribution and exposure to microwaves [11]. Although the total phenolic content of the extract decreased there was an initial increase in yield but it decreased with further increase in the amount of solvent. A reverse effect was observed for particle size. These trends may not be a direct consequence of the total phenolic content but other factors such as delivery rate of green tea extract and the ratio of ferric chloride solution to green tea extract.

Generally, almost all the ZVI particles produced did not change colour during the 12-day observation period. The particles that exhibited change in colour were

those from runs 4 and 7. This means that the green tea-solvent ratio has no significant effect on the rate of oxidation of the ZVI particles. It can be observed that a low delivery rate (8 mL/min) of the green tea extract was used for both runs 4 and 7. This may have allowed oxygen to come in contact with the particles as they are slowly formed. In run 1, the same green tea extract delivery rate was used but the total phenolic content of the extract was rather high which could have prevented the oxidation of the ZVI particles.

3.2. Effect of green tea extract delivery rate

The green tea extract delivery rate was determined to be the significant factor affecting the yield (p-value = 0.021). It can be observed from Table 2 and Fig. 2 that the higher the delivery rate, the lower is the yield of green ZVI. This correlation could be attributed to the fact that a lower delivery rate produces larger particles which tend to settle faster and hence are recovered more easily than smaller particles.

The effect of green tea extract delivery rate on particle size was found to be significant (p-value = 0.035). As the delivery rate increased, the particle size became smaller. The rate at which the reducing agent (extract) is added affects the growth of the ZVI nuclei [12]. Adequate time for particles to grow facilitates formation of ZVI clusters, thus a lower delivery rate provides enough time for nuclei formation and particle growth and a core shell structured spherical ZVI. The smallest particle size (8.82 nm) was obtained when the green tea extract delivery rate was at the highest level (17 mL/min).

3.3. Effect of $FeCl_3$-to-green tea extract ratio

The ratio of $FeCl_3$ to the reducing agent is an important factor in the synthesis of ZVI. It can be observed that the yield increased with increase in the amount of green tea extract relative to $FeCl_3$, but further increase in the amount of green tea extract resulted in a decrease in yield. On the other hand, while there was a decrease in particle size with increase in the amount of green tea extract, this is not significant (p-value > 0.05). An increase in the reducing agent to iron precursor molar ratio will affect the growth of particles in such a way as to prevent the particles from agglomeration which translates to smaller particles [13]. Since particles are smaller they are more difficult to recover, hence lower yield.

A sample TEM image of the ZVI particles illustrated in Fig. 4 shows that the synthesised NZVI appeared as nano clusters which could be due to the inherent magnetic and electrostatic properties of iron particles [14]. The nano ZVI is made up of a layered structure consisting of the metal core and oxide layer shell. Under the TEM image, the oxide layer is amorphous and has distorted layer due to the small radii of the nanoparticles [15]. It can be observed from Table 2 that all ZVI particles formed were of nano-sized. The size of the ZVI particles is comparable with those synthesized by Wang [14] using sodium borohydride as reducing agent where particle size of ZVI was less than 20 nm.

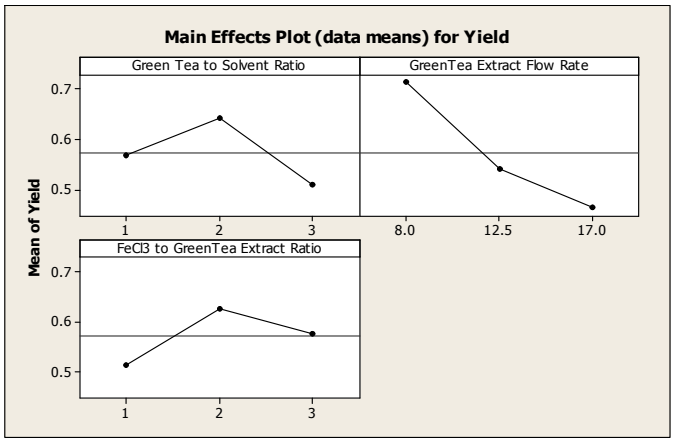

Figure 2. Main effects plot for yield.

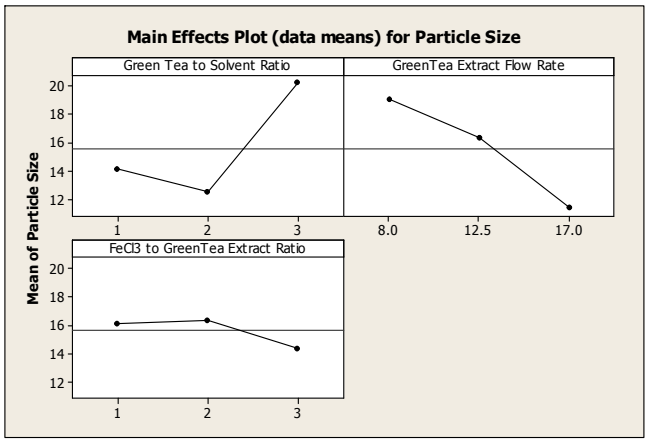

Fig. 3. Main effects plot for particle size.

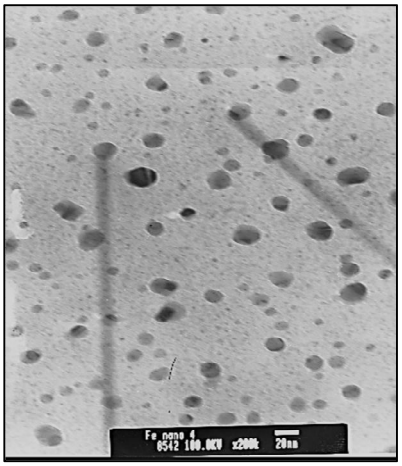

Fig. 4. Sample TEM image of ZVI particles.

3.4. Result of confirmatory run

The conditions used in the confirmatory runs were based on values of factors that gave the highest yield and smallest particle size as shown in the main effects plot (Figs. 2 and 3). The results are shown in Table 3.

Table 3. Conditions and results of confirmatory run

Factors	Yield 89.53%	Average Particle Size 8.78 nm
Green tea-to-solvent ratio (*w/v*)	1:20	1:20
Green tea extract delivery rate (*mL/min*)	8.0	17.0
$FeCl_3$ to green tea extract ratio (*v/v*)	1:2	1:3

Comparison of the results of the confirmatory run with those in the main experimental runs showed that a slightly higher yield and slightly lower particle size were obtained in the confirmatory run. These validate the results obtained in the main experimental run.

4. Conclusions

The following are the key findings of the study:

- Green ZVI was successfully synthesised using green tea extract as reducing agent producing iron nanoparticles.
- The conditions which gave the highest yield (86.48% w/w) were the following: a green tea-to-solvent ratio of 1:20, a green tea extract delivery rate of 8mL/min and a $FeCl_3$-to-green tea extract ratio of 1:2.
- The conditions which gave the smallest particle size (8.78 nm) were the following: a green tea-to-solvent ratio of 1:20, a green tea extract delivery rate of 17 mL/min and a $FeCl_3$-to-green tea extract ratio of 1:3.
- The confirmatory run showed that the yield was slightly higher and particle size slightly smaller than in the main experimental runs.
- The green tea extract flow rate was the only factor that significantly affected both the particle size and the yield.
- An inverse relationship was observed between the flow rate and the particle size and the yield.
- The green tea-to-solvent ratio did not have significant effect on the rate of oxidation.

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