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Comparison of response surface methodology (RSM) and artificial neural networks (ANN) towards efficient extraction of artemisinin from *Artemisia annua*

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ABSTRACT

The solid-liquid extraction of *Artemisia annua* remains an important source of artemisinin, the precursor molecule to the most potent anti-malarial drugs available. Industrial manufacturers of artemisinin face many challenges in regards to volatile markets and sub-optimal extraction approaches. There is a need to improve current processing conditions, and one method is to model the processing options and identify the most appropriate process conditions to suit the market forces. This study examined the impact of extraction temperature, duration and solvent (petroleum ether) to leaf proportions on the recovery of artemisinin from leaf steeped in solvent, in a central composite design (CCD), and the results were used to generate both a response surface methodology (RSM) model and an artificial neural network (ANN) model.

Appraisal of the models through the coefficient of determination (R^2) and the absolute average deviation (AAD) showed that the ANN was superior (R^2 = 0.991, AAD = 1.37%) to the RSM model (R^2 = 0.903, AAD = 4.57%) in predicting artemisinin recovery. The ANN model was subsequently used to determine the optimal extraction conditions for the recovery of artemisinin, which were found to be an extraction duration of 8 h at a temperature of 45 °C and a leaf loading of 0.12 g/ml petroleum ether, from the conditions tested. An illustration is provided in how the results obtained from an ANN model may be used to determine optimal extraction conditions in response to market conditions. In addition, a co-solvency effect has been observed between extracted impurities and petroleum ether that substantially increases the solubility of artemisinin over that in petroleum ether alone, and which will require further investigation in the future. The impact of this co-solvency effect on the efficiency of artemisinin recovery in secondary extraction cycles was found to be significant.

1. Introduction

Artemisinin-based combination therapies (ACTs) are regarded by the World Health Organisation (WHO) as the most important class of drugs available in the fight against malaria, which claimed an estimated 660,000 lives in 2010 (WHO, 2013a). The critical starting material for all ACTs is artemisinin, which is primarily obtained through the solid-liquid extraction of the leaves of the *Artemisia annua* plant. An alternative source of artemisinin has recently been approved for use in ACTs (WHO, 2013b). This new source is from a genetically modified yeast that over produces artemisinic acid, which is then isolated and photocatalytically converted to artemisinin (Paddon et al., 2013). However, it is expected that the short to medium-term demand will still have to be met by *A. annua* extraction (A2S2, 2012). This leaves the artemisinin industry in a precarious position, with farmers disinclined to plant further crops, and industrial manufacturers facing numerous difficulties comprising volatile markets, variable biomass feedstock quality, unrecovered value in waste streams and technological limitations. There is a need for industrial manufacturers to optimise current approaches, thereby improving the profitability of production and ensuring a sufficient supply of artemisinin.

A manufacturing process cannot be optimised without first knowing the process details and such information is held by industry to retain market advantage. However, some heuristic rules can be provided for industry to review and apply for achieving their

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own particular commercial objectives. Current industrial extractions use hexane, petroleum ether, toluene and HFC-134a, with petroleum ether being the most common solvent but demonstrating insignificant difference in performance to hexane (Christen and Veuthey, 2001; Lapkin et al., 2006; Vandenberghe et al., 1995). The solubility of artemisinin in a range of other solvents has been examined, which might lead to improved extraction of artemisinin (Lapkin et al., 2010; Liu et al., 2009; Nti-Gyabaah et al., 2010) due to their higher affinity to artemisinin, but they have not reached commercial application. This could be due to difficulties in the supply of sufficient quantities for extraction, increased cost implications and the increased risks associated with some solvents (acetonitrile, toluene, chloroform, etc.). The use of ethanol as an extraction solvent has been investigated by Fleming and Von Freyhold (2007) and, whilst positive results were obtained, the extracts are likely to contain higher quantities of sugars and polar impurities that hinder the subsequent crystallisation of artemisinin from the extract mixture (Lapkin et al., 2010). Other potential drawbacks to the industrial use of ethanol include a higher latent heat of vapourisation over petroleum ether that makes solvent recovery more expensive, in addition to the miscibility of ethanol and water that would result in the need for distillation to re-concentrate the ethanol after steam stripping of residual solvent in the extracted leaf bed.

Extraction is undertaken either by submerging the leaves in the extracting solvent (with or without agitation, which includes horizontal tumbling) or percolating the solvent through the leaf bed. The temperature range for extraction is 30–45 °C and the process may be undertaken over durations ranging from 8 to 48 h, with the possibility of additional extraction cycles to improve artemisinin recovery (Brisibe et al., 2008; Lapkin et al., 2006). Ethyl acetate may be added to the hexane/petroleum ether up to 5% by volume to increase the extent of artemisinin extraction (Brisibe et al., 2008) and to reduce the possibility of explosion through static build up and discharge (Lapkin et al., 2010). The proportion of leaf to solvent used by industry is difficult to ascertain from the literature, though it has been suggested that one kilogram of leaf could be extracted with one litre of solvent (Brisibe et al., 2008); such an approach would be impractical without the use of solvent percolation because the low density of dry biomass would ensure that the leaf bed greatly exceeds the level of extracting solvent.

With such a wide range of conditions reported in the literature, the task of process optimisation can only be undertaken using a methodology that can assess the individual impact of each process condition on overall efficiency. One such approach is the response surface methodology (RSM) developed by Box and Wilson (1951), which has seen wide application in the chemical industry due to its ability to optimise a process with a minimal amount of experimental data. As a statistical tool, RSM can model the impact of various process factors, both individually and through their cumulative interactions, on a system response, thereby providing an indication of the optimal operating region (Box et al., 2005). More recently, artificial neural networks (ANN) are finding increasing use as predictive tools in an extensive range of disciplines, including engineering, due to their ability to employ learning algorithms and discern input–output relationships for complex, nonlinear systems (Alavala, 2007; Zobel and Cook, 2011). Both RSM and ANN have been applied to optimise a range of natural product extraction processes and the resultant models show a strong correlation with experimental results. Recent examples include the extraction of phenols from mangosteen hull (Cheok et al., 2012), essential oils from *Diplotaenia cachrydifolia* (Khajeh et al., 2012), coumarin from *Cuscuta reflexa* (Mitra et al., 2011), secoisolariciresinol diglucoside from flaxseed (Nemes et al., 2012), oils from *Orthosiphon stamineus* (Pouralinazar et al., 2012) and *passiflora* seeds (Zahedi and Azarpour, 2011) and the extraction of natural dyes (Sinha et al., 2012, 2013).

The aim of this investigation was to develop and compare RSM and ANN models to indicate how the percentage recovery of artemisinin, through the extraction of *A. annua* using petroleum ether, might be optimised. The parameters investigated comprise solvent temperature, extraction duration and the proportion of leaf to solvent, in an extraction process that is considered to approximate the industrial approaches. The generated models were then compared in their suitability for predicting artemisinin recovery by analysing their coefficient of determination (R^2) and absolute average deviation (AAD) from experimental data. In the case of RSM, ANOVA was applied to assess any significant lack of fit with the experimental data. The models were then used to determine the impact of the extraction conditions on artemisinin recovery, thereby providing an indication of the optimal approach. The ANN model was then used to illustrate how processing conditions might be altered in order to optimise the first extraction cycle in response to market pressures. For the two case study conditions examined, an additional second extraction cycle was then performed to inform on the potential of improving the recovery of artemisinin further.

2. Materials and methods

2.1. Characterisation of A. annua

Samples of *A. annua* harvested from Wanzhou, Chongqing, China were supplied pre-milled (<2.80 mm) and dried by PIDI Standard (Holdings) Ltd (Guangzhou, Guangdong, China). The moisture content was determined to be 7.9 ± 0.2 wt% (n = 3) by drying to constant weight at 105 °C. The tapped bed density of the biomass was determined to be 0.21 ± 0.006 g/ml (n = 3) by filling and manually tapping a 50 ml measuring cylinder to provide an indication for the maximum solvent to leaf proportions that would be practical for extraction without solvent percolation.

The artemisinin content of the biomass was determined using a modified version of the method presented by Van Nieuwerburgh et al. (2006), using increased sample size and an extended extraction duration. Approximately 1.667 g of biomass was accurately weighted in triplicate and contacted with 10 ± 0.1 ml of chloroform (laboratory reagent grade, Fisher Scientific, UK) in 60 ml Boston type bottles, which were sealed and placed on a reciprocating shaker table operating at 170 rpm for a period of 5 min. After this duration, the supernatants were decanted and filtered to 0.2 μm using PTFE filter syringes (Fisher Scientific, Loughborough, UK), with 3 ml aliquots taken to dry down under atmospheric conditions (17 ± 1 °C). Filtration of artemisinin standard solutions using this methodology confirmed that there was no detectable decrease in artemisinin concentration due to adsorption onto the filter membrane. Prior to HPLC–UV analysis by a methodology used previously (Pilkington et al., 2012), the samples were reconstituted for a period of 24 h on a reciprocating shaker table operating at 170 rpm. The artemisinin content was found to be 1.37 ± 0.06 wt% of dry leaf.

2.2. Experimental design

The extraction parameters of solvent temperature (X_1), duration (X_2) and solvent to leaf proportions (X_3) were investigated for their impact on the recovery of artemisinin from *A. annua* using petroleum ether. Recovery is presented as the weight percentage of artemisinin detected in the extract mixture when compared to the total artemisinin present in the dry biomass. The temperature range of investigation was chosen to be 30–45 °C in accordance with the values published in the literature when hexane or petroleum