Iranian Journal of Chemical Engineering
Vol. 11, No. 4 (Autumn 2014), IAChE

Effect of Ethanol Concentration in Ultrasound Assisted Extraction of Glycyrrhizic Acid from Licorice Root

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Abstract

The major active component in licorice is glycyrrhizic acid which is very useful in the pharmaceutical industry. In the present work, the extraction of glycyrrhizic acid in licorice is carried out in a stirred reactor as well as under indirect sonication in an ultrasonic bath. Batch extraction is carried out in a glass reactor of 150 mL capacity equipped with a six bladed glass turbine for agitation. Ultrasound assisted extraction has been carried out in a dual frequency ultrasound cleaning bath. The glycyrrhizic acid is analyzed using HPLC. Various process parameters such as solvent concentration, power, frequency of ultrasound, extraction temperature and solvent to solute ratio, which affect the extraction yield are optimized for both techniques. In ultrasound assisted extraction with final optimized conditions, i.e. 50% ethanol as solvent, 230 W power, 40:1 solvent to solute ratio, 30ºC temperature and 40 kHz frequency, 95.69% extraction is obtained in 20 minutes, whereas in extraction using stirred reactor only 68% extraction is observed in 60 minutes. Kinetic of the extraction process is studied and volumetric mass transfer coefficients as well as theoretical yield for different process parameters are estimated.

Keywords: *Licorice, Glycyrrhizic Acid, Ultrasound, Kinetics, Optimization*

1. Introduction

Leaching or solid-liquid extraction is concerned with the extraction of a soluble constituent from a solid by means of a solvent and this is one of the important steps in the isolation and separation of valuable natural ingredients from plant. The pharmaceutical industry and other industries like food, flavor, cosmetic and dyes deal with the recovery of active principles from plants. Traditionally, these ingredients are extracted by solvent extraction which is carried out by supplying energy either by heating or agitation. But the major drawbacks of these techniques are requirements of high energy, high solvent and time. So, this leads to the need for development of new efficient extraction techniques which can overcome the disadvantages of traditional techniques so that the process of extraction can be commercialized.

Ultrasound is used in different operations in chemical engineering such as waste-water treatment, drying, sonochemistry and solid–liquid extraction [1]. The use of an

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ultrasound in the extraction of natural products such as linalool from citrus flower [2], essential oil from garlic [3], lycopene from tomato [4], hesperidine and other flavonoids from citrus peel [5] has been reported to increase extraction rate and significantly reduce extraction time. In ultrasound assisted extraction, the extraction rate is enhanced due to cavitation. The cavity developed grows in size and then collapses abruptly with the release of energy at a massive rate, thus increasing the local temperature and pressure. Therefore, greater penetration of solvent into cellular material occurs and this improves the release of cell content into bulk medium. Similarly, the physical effects such as liquid circulation and turbulence produced by cavitation help in increasing the contact surface area between the solvent and targeted compounds by permitting greater penetration of solvent into the sample matrix. Therefore the main advantages of ultrasound-assisted extraction include the reduced extraction time with less solvent consumption [6]. In addition, ultrasound-assisted extraction can be carried out at a lower temperature which helps avoid thermal damage to the extracts, so ultrasound can be used for the extraction of heat labile products [7].

Licorice is extensively used in herbal medicines worldwide due to its anti-inflammatory, anti-viral, anti-allergic, anti-oxidant, and anti-cancerous properties. Traditionally it is used for the treatment of peptic ulcers, asthma, pharyngitis, malaria, abdominal pain, insomnia, infections. The major active component in licorice is glycyrrhizic acid while other important constituents include flavonoids, isoflavonoids, chalcones, coumarins, triterpenoids, sterols, starch, sucrose and glucose, lignins, amino acids, amines, gums, volatile oils [8]. Besides medicinal usage as an ingredient in pharmaceutical preparations, GA is also used extensively as a sweetener or functional additive in tobacco, food and confectionery products.

In our previous study we carried out the extraction of glycyrrhizic acid (GA) from licorice root powder using water as a solvent [9]. But it is reported in the literature that GA also has good solubility in ethanol. Hence, here it is proposed to carry out ultrasound assisted extraction of GA from licorice using absolute ethanol as well as different ethanol-water compositions. The parameters which affect the yield in ultrasound assisted extraction process are extraction time, ethanol concentration, power, ultrasound frequency, extraction temperature and solvent to solute ratio. The main objective of this work is to develop the optimized ultrasound assisted extraction process for glycyrrhizic acid from licorice root and compare this with conventional methods. The second objective is to investigate the kinetic of this extraction process.

2. Materials and methods

2-1. Material

Licorice powder is purchased from a local market. Water used is freshly prepared de-ionized water from Millipore Milli–Q 50. Ethanol used is of AR grade purchased from Hi Media Ltd., Mumbai, India. Acetonitrile and acetic acid used as solvent for High Pressure Liquid Chromatography were analytical grade purchased from Hi Media Ltd., Mumbai, India. Standard, i.e.

Glycyrrhizic acid (mono-ammonium salt hydrate ≥ 70 %) was purchased from Aldrich Chemical Company, USA.

2-2. Apparatus

Ultrasound assisted extraction has been carried out in a dual frequency ultrasound cleaning bath (Model 6.5l200 H, Dakshin, India) of internal dimensions 230 x 1500 x 150 mm and tank capacity approximately 6.5 liters, with an ultrasonic power of 200 watts and frequencies of 25 kHz and 40 kHz, equipped with heater and digital temperature controller/indicator. A selector switch is provided on the panel to select one operating frequency at a time. Power variation is possible by varying input AC voltage through auto-transformer.

2-3. Ultrasound assisted extraction

The extraction is carried out in a flat bottom glass vessel kept in an ultrasound bath. The clearance of the glass vessel from the bottom of the bath (where transducers are fixed) is kept constant throughout the experiments. The measured quantity of the licorice powder is placed in a glass vessel and the required amount of solvent is added. In order to avoid the loss of solvent, the glass vessel is covered with a lid before it is put into the ultrasound bath. Then the ultrasonication is done. Samples are withdrawn at specific time intervals and then filtered to obtain a clear extract which is further diluted and analyzed using HPLC. Extraction experiments are carried out to study the effect of parameters which affect the extraction yield such as solvent concentration, power, ultrasonic frequency, extraction temperature and solvent to solute ratio. The ultrasound bath is provided with heater and temperature controller. In order to study effect of temperature, the water in ultrasound bath is heated using the provided heating arrangement to desired set point. The temperature inside the glass vessel is measured by the thermocouple inserted in it. There is a difference of 7-8°C in bath and vessel temperature. Hence the bath temperature is kept 8°C above the desired vessel temperature. Experiments are carried out for 20 min so there is a rise of 1-2°C in bath temperature which is constant in all experiments.

2-4. Soxhlet extraction

Extraction is carried out in a conventional Soxhlet apparatus which consists of distillation flask placed in oil bath, thimble holder and the condenser. The licorice powder is packed in filter paper and placed in thimble holder which is filled with the condensed fresh solvent from the distillation flask. The extraction of GA takes place in the thimble chamber. When the liquid reaches the overflow level, the liquid moves through the siphon and unloads it back into the distillation flask, carrying extracted solutes into the bulk liquid. In solvent flask, solute is separated from the solvent by distillation. Solute is left in the flask and fresh solvent passes back into the solid bed material. The operation is repeated until complete extraction is achieved.

2-5. Batch extraction

Batch extraction is carried out in a glass reactor of 15 mL capacity equipped with a six bladed (pitched blade) glass turbine for agitation. The measured quantity of the licorice powder is taken in a glass reactor and required amount of solvent is added to it. Mixture is agitated for 60 minutes. Samples are withdrawn at specific time interval and filtered. The clear liquid samples are then diluted and analyzed for GA using HPLC. Different parameters affecting the extraction such as extraction time, solvent to solute ratio, speed of agitation and extraction temperature are optimized and the final extraction experiment at the optimized conditions is carried out to be compared with ultrasound assisted extraction.

2-6. Analytical method

The glycyrrhizic acid is analyzed using HPLC. The HPLC instrument is of Thermo Scientific which consists of Solvent degassing unit Spectra System SCM 1000, gradient HPLC pump Spectra System P4000 and Spectra System UV 2000 as a UV detector. The column used is C18 of dimensions 125 x 4.6 mm. The wavelength is set at 254 nm. The mobile phase is 70% methanol and 30% acidified water (1% acetic acid) and the flow rate is 1 ml/min.

3. Results and discussion

3-1. Effect of concentration of ethanol

The effect of ethanol concentration on the extraction of glycyrrhizic acid from licorice is studied and the results are shown in Fig. 1. Concentration of ethanol is varied from 10% to 100%. It is found that the % extraction increases up to 50% ethanol concentration. Thereafter, it remains constant till 70% ethanol concentration and subsequently decreases. When 100% ethanol is used as a solvent a significant decrease in % extraction has been observed. Water is required for the swelling of the cellular material, so that the permeability of cell wall increases and they can break easily upon introduction of ultrasound. At 100% ethanol as there is no water available for swelling of cells, extraction yield is very low. Hence, 50% ethanol concentration is considered optimum and used in all the further sets of experiments.

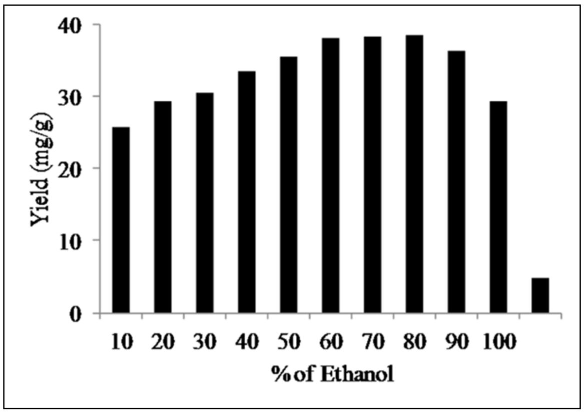

Figure 1. Effect of concentration of ethanol on extraction yield of GA (Power: 200 W, Frequency: 25 kHz, solvent to solute ratio: 1:30, temperature: 30°C).

3-2. Kinetic of ultrasound assisted extraction

The extraction kinetics of ultrasound assisted extraction of glycyrrhizic acid from licorice root is studied using 50% ethanol as a solvent. The change in the amount of GA extracted per g of licorice with time is shown in the Fig. 2. The curve obtained follows the same nature as that of classical extraction from plant material. Two different rates of extraction can be observed. The first is the fast rate obtained initially which is followed by the gradual increase in the concentration of GA in the extract. It can be seen from the Fig. 2 that the rate of extraction of glycyrrhizic acid is higher, initially up to 6 min. Thereafter there is very gradual increase in extraction and after 20 min no significant increase is observed in the extraction of GA. Kinetic model proposed by Liauw *et al.* [10] is used to study the kinetic of this process. The assumption made for this model is that the mechanism which controls the rate of GA extraction is the mass transfer of GA from licorice root powder to the bulk of the solvent.

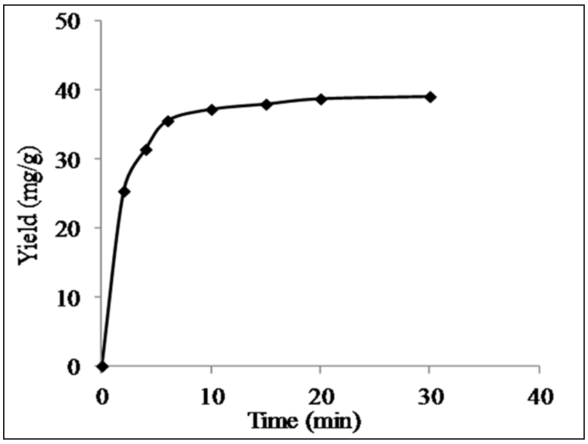

Figure 2. Effect of time on extraction yield of GA (Power: 200 W, Frequency: 25 kHz, solvent to solute ratio: 1:30, temperature: 30°C).

The mass transfer rate of GA from licorice root powder can be written as:

$$\frac{dW_A}{dt} = k.A.(C_{Ai} - C_A) \quad (1)$$

Where dW_A/dt is the mass transfer rate of GA (mg/g.s), C_A and C_{Ai} are the concentration of GA in liquid at time t and at equilibrium. Here k is mass transfer coefficient and A is surface area for mass transfer process. Since the extraction was performed in a batch process and its volume was kept constant during the process,

$$\frac{dW_A}{dt} = k.\frac{A}{V}.(W_{Ai} - W_A) \quad (2)$$

$$\frac{dW_A}{dt} = k.a.(W_{Ai} - W_A) \quad (3)$$

Where, *k.a.* is volumetric mass transfer coefficient (s^{-1}). To solve the equation the following boundary conditions are used:
At the beginning of the extraction process t = 0, the mass of GA in bulk liquid, i.e. $W_A = 0$
At any time t, mass of GA in bulk liquid is $W_A = W_{Ai}$
Using these boundary conditions, integrating eq (3) gives the following result:

$$W_A = W_{Ai}[1 - \exp(-k.a.t)] \quad (4)$$

This equation is rearranged so that it can be written in terms of yield per unit mass of licorice powder.

$$Y_A = Y_{Ai}[1 - \exp(-k.a.t)] \quad (5)$$

Where, Y_A and Y_{Ai} are yield of GA at time t and at equilibrium per mass of licorice powder (mg of GA/ g of licorice) respectively.
The values of GA yield at equilibrium Y_{Ai} and volumetric mass transfer coefficient $k.a$ at different operating parameters are estimated by the nonlinear least square fit of the eq (5) to experimental data. Then these values are used for the prediction of extraction yield of GA for different operating parameters.

3-3. Optimization of process parameters and validation of model

3-3-1. Effect of power

Since power is an important parameter in ultrasound assisted extraction, its effect on extraction has been investigated. The power is varied from 120 W to 200 W and the results obtained are shown in the Fig. 3. The final % extraction obtained is the same for all the 3 power inputs, but the rate of extraction is affected significantly. It is observed that the extraction rate increases with an increase in power input. Therefore, 30 minutes is required to get 75% extraction at 120 W, whereas the same extraction was obtained in 10 minutes at 200 W. Therefore, the % extraction per unit energy is calculated for all the three power inputs and it is highest for 200 W power. Hence, all the further experiments are carried out at 200 W.

By applying the kinetic model described above for this experimental data, values of $k.a$ and Y_{Ai} are calculated by using nonlinear least square fit method. These values are tabulated in Table 1. It can be seen from the table that the values of volumetric mass transfer coefficient increases with an increase in the power. This indicates that the rate of mass transfer is higher at higher powers. These values are then used to predict the yield of the GA at different time. In Fig. 3, experimental and predicted yield of GA is plotted where bullets represent the experimental data and solid line represents the predicted yield. It can be seen from Fig. 3 that there is good agreement between experimental and predicted yield of GA.

Table 1
Effect of power on volumetric mass transfer coefficient and yield of GA.

Power	$k.a$	Y_{Ai} Experimental	Y_{Ai} Predicted	RMSD
120 W	0.111	35.35	35.51	0.16
160 W	0.17	36.99	36.8	0.19
200 W	0.21	39.08	40.04	0.96

3-3-2. Effect of ultrasound frequency

Extraction of licorice has been carried out at two different frequencies, i.e. 25 kHz and 40 kHz to investigate its effect on % extraction. Results are shown in Fig. 4 and it is observed

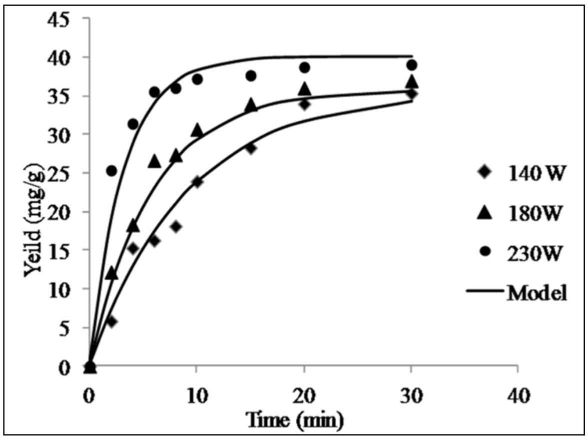

Figure 3. Effect of Power on extraction yield of GA (Frequency: 25 kHz, solvent to solute ratio: 1:30, temperature: 30°C).

That % extraction at 40 kHz is higher than that at 25 kHz. At higher ultrasonic frequency the cavitation is higher due to which the mass transfer from bulk cell material to the solvent is enhanced. Similar increase in extraction at higher frequency up to 45 kHz is also reported for extraction of salvianolic acid B from Salvia miltiorrhiza root [11]. Therefore frequency of 40 kHz has been considered to be an optimum frequency. The values of $k.a$ and Y_{Ai} for different frequency were predicted from kinetic model and are presented and plotted in Table 2 and Fig. 4 respectively, showing satisfactory agreement with experimental values. Higher values of k.a at higher frequency represent the greater mass transfer rate at 40 kHz frequency than 25 kHz.

Table 2
Effect of frequency on volumetric mass transfer coefficient and yield of GA.

Frequency	$k.a$	Y_{Ai} Experimental	Y_{Ai} Predicted	RMSD
25 kHz	0.199	38.8	38.86	0.06
40 kHz	0.204	41.62	42.09	0.47

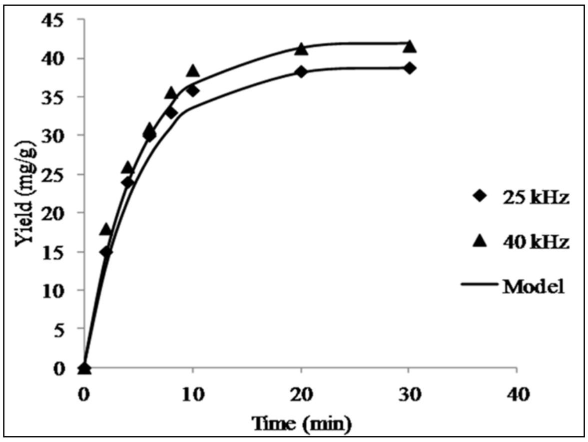

Figure 4. Effect of Frequency on extraction yield of GA (Power: 200 W, solvent to solute ratio: 1:30, temperature: 30°C).

3-3-3. Effect of temperature

Temperature has a positive influence on the solubility of GA, hence it was expected that the extraction will increase with temperature. Therefore extraction is carried out at different bath temperatures, i.e 30°C, 50°C and 70°C and results are shown in Fig. 5. But it is observed that there is no significant change in % extraction of GA with increase in temperature. With increase in the temperature, vapor pressure increases. Vapor pressure has a great influence on the occurrence and the intensity of acoustic cavitation. When the temperature is low bubbles are few but they collapse with relatively high intensity which enhances the cell disruption. But, when the temperature is high, although more bubbles are created, they collapse with less intensity. Surface tension also decreases with the increase of temperature which affects the bubble formation and collapse. Thus, at higher temperature bubbles collapse with less intensity and reduce the mass transfer enhancement [12]. Hence, the counterbalance of increase in the extraction due to increased solubility and decreased mass transfer due to less intensive cavitation bubble collapse at higher temperature results in the marginal change in extraction of GA.

The estimated model values in comparison with experimental values are represented in Table 3 and plotted in Fig 5. From Table 3 it can be seen that the values of *k.a* increase with the rise in temperature which is because of the higher mass transfer rate at elevated temperature. Increase in mass transfer rate is an effect of increased solubility at higher temperature. It can be seen from Fig. 5 that there is good agreement between experimental and predicted values of the yield of GA.

Table 3
Effect of temperature on volumetric mass transfer coefficient and yield of GA.

Temperature	*k.a*	Y_{Ai} Experimental	Y_{Ai} Predicted	RMSD
30	0.204	42.66	41.26	1.4
50	0.211	43.41	43.38	0.03
70	0.216	44.17	44.7	0.53

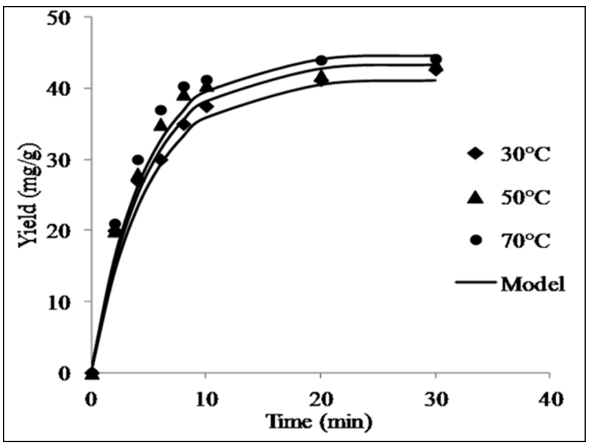

Figure 5. Effect of temperature on extraction yield of GA (Power: 200 W, Frequency: 40 kHz, solvent to solute ratio: 1:30).

3-3-4. Effect of solvent to solute ratio

The solvent to solute ratio affects % extraction significantly. Therefore, this ratio is varied from 10:1 to 50:1 and results are shown in Fig. 6. It is observed that the % extraction increases with an increase in solvent to solute ratio up to a certain level and then it decreased for the higher solvent to solute ratio. When solvent to solute ratio is higher, it provides the larger concentration gradient which favors the mass transfer. So the % extraction is increased when solvent to solute ratio is increased from 10:1 to 40:1. The solvent volume in 40:1 solvent to solute ratio is sufficient to extract the solute and solvent becomes saturated. Therefore, when 50:1 solvent to solute ratio is used, the solution gets diluted and this is the reason for lower % extraction at this ratio. As the highest % extraction is obtained at 40:1 solvent to solute ratio, this ratio is considered as an optimum ratio.

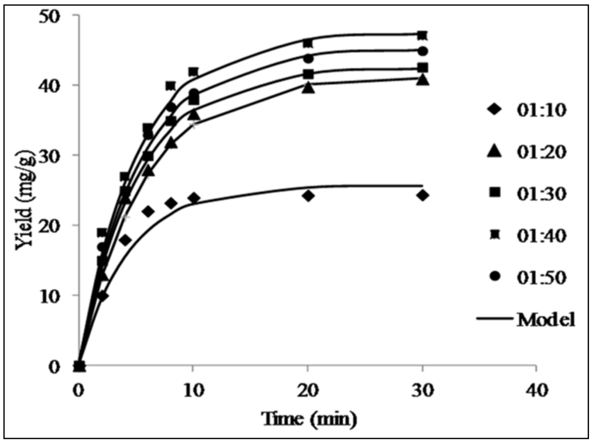

Figure 6. Effect of solvent to solute ratio on extraction yield of GA (Power: 200 W, Frequency: 40 kHz, temperature: 30°C).

Values of *k.a* and Y_{Ai} estimated from kinetic model are listed in Table 4. It can be clearly observed from Fig. 6 that the agreement between the experimental observations and the predicted values from the kinetic model is quite good. From Table 4 it is clear that the values of *k.a* increase with the increase in solvent to solute ratio up to 40:1 ratio and then decrease further which means that the rate of mass increases up to 40:1 ratio. Mass transfer rate is higher up to 40:1 ratio because the concentration gradient increases with the addition of solvent but the further decrease in the mass transfer rate at 50:1 ratio indicates that 40:1 ratio is sufficient for leaching and the addition of more solvent after this does not affect the mass transfer rate.

Table 4
Effect of temperature on volumetric mass transfer coefficient and yield of GA.

Solvent: Solute	*k.a*	Y_{Ai} Experimental	Y_{Ai} Predicted	RMSD
10:01	0.228	24.40	25.71	1.31
20:01	0.181	41.00	41.26	0.26
30:01	0.194	42.66	42.52	0.14
40:01	0.196	47.19	47.46	0.27
50:01	0.192	45.01	45.15	0.14

3-4. Comparison of ultrasound assisted extraction with conventional method

Glycyrrhizic acid is extracted from licorice by UAE as well as conventional soxhlet extraction and batch extraction in order to evaluate effects of ultrasound on the extraction efficiency. Table 5 shows the optimum results respectively with the soxhlet extraction, batch extraction and ultrasound assisted extraction. Soxhlet extraction has taken 300 minutes to extract 100% GA from licorice sample while UAE required only 20 minutes to achieve 95.68% extraction. Also, the soxhlet extraction requires heating up to 100°C, whereas in UAE the extraction is carried out at room temperature. With batch extraction only 68.06% extraction is obtained in 60 minutes time. Therefore, the conventional extraction took more time and consumed more solvents. Fang Chen *et al*. have also reported the same results [13]. Chen *et al*. (2007) carried out microwave-assisted micellar extraction of GA and achieved 98.4% recovery of GA in 5 min time, which is comparable with our result [14].

Table 5
Comparison of soxhlet extraction, UAE and batch extraction.

Method	Time (min.)	Temperature (°C)	Solvent to solute ratio (ml/g)	% Extraction
Soxhlet Extraction	300	100	50:1	100
Ultrasound assisted Extraction	20	30	40:1	95.69
Batch extraction	60	30	40:1	68.06

4. Conclusions

In the present work, extraction of glycyrrhizic acid from licorice root is successfully carried out using ultrasound. Process parameters which affect the extraction yield viz. solvent concentration, power, ultrasound frequency, temperature and solvent to solute ratio are investigated. Concentration of ethanol affects the % extraction and 50% ethanol concentration is the optimum value. Although the final % extraction obtained at various power input is the same, the rate of extraction is affected significantly with variation of power. The highest extraction rate is obtained at 200 W. Extraction is increased with increase in solvent to solute ratio up to 40:1. It is also observed that there is no effect of temperature on the % extraction of GA and with the increase in the frequency of ultrasound there is a slight increase in % extraction. In ultrasound assisted extraction with final optimized conditions, i.e. 50% ethanol as solvent, 230 W power, 40:1 solvent to solute ratio, 30ºC temperature and 40 kHz frequency, 46.52 mg/g yield, 95.69% extraction is obtained. The % extraction obtained with ultrasound assisted extraction is compared with that of soxhlet extraction and batch extraction. Ultrasound assisted extraction gives higher % extraction as compared to batch extraction and requires less time compared with both soxhlet and batch extraction. So it can be concluded that the ultrasound assisted extraction of glycyrrhizic acid from licorice is an effective way for extraction with advantages of lower time, lower heating requirement and higher % extraction. The values of predicted yields using kinetic model show good agreement with the experimental data for all parameters.

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