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Aqueous Hydrotropic Solution as an Efficient Solubilizing Agent for Andrographolide from *Andrographis paniculata* Leaves

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Aqueous Hydrotropic Solution as an Efficient Solubilizing Agent for Andrographolide from *Andrographis paniculata* Leaves

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Abstract: An aqueous solution based extraction process for andrographolide from *Andrographis paniculata* leaves has been developed using alkyl benzene sulfonates and carboxylates as hydrotropes. The plant cells are permeabilized by the hydrotrope solutions followed by solubilization of andrographolide into the solutions. The extraction and solubilization of andrographolide is affected by structure and concentration of hydrotrope, temperature and particle size. Sodium cumene sulfonate (Na-CS) shows the most efficient solubilization of andrographolide amongst the hydrotropes studied. The solubility of andrographolide increased by two orders of magnitude in Na-CS aqueous solutions and ~96% andrographolide extraction was achieved in just 20 min.

Keywords: Andrographolide, *Andrographis paniculata*, hydrotropy, sodium cumene sulfonate, sodium salicylate, extraction

INTRODUCTION

Andrographis paniculata Nees (*Acanthaceae*), contains andrographolide (Fig. 1) as the major constituent and andrographiside, deoxyoxoandrographolide, and neoandrographolide as minor constituents (1, 2). Andrographolide is widely used as a hepatoprotective agent. It also shows choleretic, antidiarrhoeal, immunostimulant, and anti-inflammatory activities (2–4).

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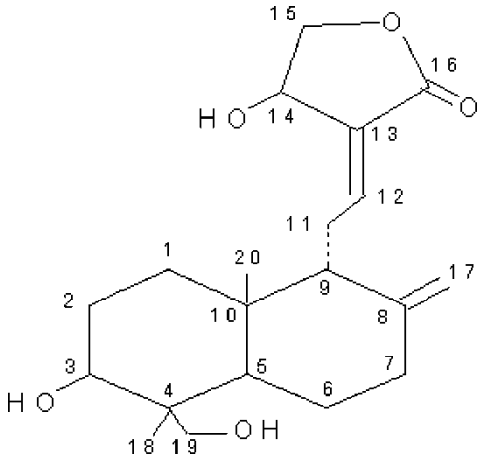

Figure 1. Structure of andrographolide.

Conventionally, isolation of andrographolide involves extraction from defatted pulverized leaves by alcohol or other polar organic solvents followed by its purification by crystallization (5, 6). The use of large volumes of volatile and inflammable solvents and labor-intensive solid handling in large quantities make the process unattractive.

Our present report deals with an aqueous solution based extraction process for andrographolide using hydrotropes. The hydrotropic phenomenon has been reported for successful extraction of curcuminoids from turmeric (7), piperine from black pepper (8), boswellic acids from *Boswellia serrata* resins (9) and diosgenin from *Dioscorea* rhizomes (10). Hydrotropes are highly water-soluble small molecular weight organic salts and show strong concentration dependence for their ability to dissolve other organic compounds in aqueous solutions. The hydrotropic extraction processes usually involve recovery of the product by dilution with water as the solubility of a solute is an exponential function of the hydrotrope concentration and the solute is separated as another phase on dilution. The dilute hydrotrope solution needs to be concentrated in order to recycle it back to the extraction stage. This evaporation of water adds substantially to cost of the process. Certainly the higher cost of the product such as andographolide, so recovered by dilution, can justify the cost of concentrating the diluted solutions either by evaporation or even by membrane processes.

In this paper we have proposed the recovery of the product from the hydrotrope solutions by partitioning with an organic solvent as an alternative to dilution by water. We experimentally explore the extraction of andrographolide from *Andrographis paniculata* leaves using aqueous hydrotropic solutions followed by its partitioning into organic solvents. The major advantage of the approach is recovery of andrographolide in a much pure form.

Hydrotropes have been extensively investigated as drug solubilizers (11). Particularly carboxylate salts, such as Na-salicylate, are major hydrotropes

used in drug formulations (12). Na-Ibuprofen is also one of the most efficient hydrotropes (13). They have also been used as coupling agents to solubilize water insoluble and often incompatible functional ingredients of household and institutional cleaning products and personal care products. Further, hydrotropes are readily biodegradable under aerobic conditions (14). Because of their own very high water solubility, simple water washing can remove hydrotropes from the product. The final purification of most of the products such as andrographolide, however, has to be done by adsorption and/or chromatography operations. We have given more emphasis here on primary extraction of water insoluble active ingredients using aqueous solutions of hydrotropes which should avoid use of large amounts of volatile organic solvents and/or expensive high pressure units.

MATERIALS AND METHODS

All the solvents and chemicals used were of analytical grade. Dried pulverized leaves of *Andrographis paniculata* (*Acanthaceae*) of with the maximum moisture content of $\sim$1.5% were obtained from M/s Natural Remedies Ltd., Banglore, India. The raw material was first pulverized and the dry powder was separated by mechanical sieving into different average particle size batches, i.e., 2.8–3 mm, 0.8–1 mm, 0.25–0.4 mm and 0.1–0.2 mm, respectively. The hydrotropes, sodium salts of cumene sulfonate (Na-CS), and *p*-toluene sulfonate (Na-PTS), were purchased from Navdeep Chemicals Ltd., Mumbai and were recrystallized from methanol and dried before use. Sodium salicylate (Na-Sal) was obtained from Swastik Pharmaceuticals, Mumbai. Pure andrographolide was isolated by the reported procedure (5) and subsequently characterized by IR and NMR whose values are given in Appendix I.

Analytical Methods

The analysis was performed using high performance liquid chromatography (HPLC) with 250 mm long Hi- QSil- C-18 column. The column was initially rinsed with methanol for 30 min and then equilibrated with methanol-water (65:35) mobile phase. The column was mounted on a Jasco Pu-2080 plus HPLC chromatograph equipped with a 20-μL loop injector and a MD-2010 photodiode detector. The mobile phase flow rate was maintained at 1.0 cm^3/min and the detection wavelength was 223 nm. The analysis was isocratic and was carried out for 12 min^2.

Solubility Measurements

The solubility measurements were carried out in a cylindrical glass vessel of maximum volume 50 cm^3 equipped with a six bladed turbine impeller

(*i.d.* 2 cm). This entire assembly was kept in a constant temperature water bath of accuracy $\pm 0.1^{\circ}C$ during the solubility studies. The solubility studies were conducted by suspending pure andrographolide in aqueous hydrotrope solutions of concentration in the range $0.05-2.0\,mol/dm^3$. The solution was equilibrated with an excess amount of pure andrographolide under vigorous stirring conditions at constant temperatures ranging from $20^{\circ}C$ to $90^{\circ}C$. After 4 hr, the stirring was stopped and the suspension was allowed to settle for 15 min. A glass pipette with its tip protected by a microfilter was used to withdraw clear upper portion of the solution. The sample was analyzed to determine its andrographolide content.

Kinetics of Extraction

The extraction of andrographolide was conducted in a fully baffled cylindrical glass vessel ($100\,cm^3$) equipped with a six- bladed turbine impeller (2 cm *i.d.*). The pulverized leaves of *Andrographis paniculata* were suspended in aqueous solutions of hydrotrope of different concentrations upto $2.0\,mol/dm^3$. The suspension was agitated vigorously at 1200 rpm. The rate of extraction, as followed by the andrographolide concentration in the solution, became independent of the speed of agitation beyond 1000 rpm. Samples were withdrawn from the solution using a microfilter fitted pipette after definite time intervals and analyzed for the andrographolide concentration.

The total andrographolide content in the raw material was determined separately by continuous soxhlet extraction with methanol. The raw material prior to the extraction was defatted using petroleum ether (b.p. $40^{\circ}C-60^{\circ}C$) for 18 hr. The methanol extraction was carried out for 48 hr and the total andrographolide content was estimated to be 2.3% (w/w) in the leaves.

RESULTS AND DISCUSSION

Figures 2–4 show solubility of andrographolide in aqueous solutions of different hydrotropes as a function of temperature. In water the solubility of andrographolide varies from $0.035\,gm/dm^3$ to $0.181\,gm/dm^3$ when the temperature was increased from $20^{\circ}C$ to $90^{\circ}C$. In the hydrotrope solutions also, the solubility of andrographolide increases with temperature at a given hydrotrope concentration and also with hydrotrope concentration at a given temperature. An increase of two orders of magnitude in the solubility of andrographolide was observed in aqueous Na-CS solutions.

Hydrotropy has been claimed to be a collective molecular phenomenon and the self-aggregation of hydrotrope molecules is considered a prerequisite for the enhanced solubility of a solute in aqueous hydrotrope solutions (15–17). A hydrotrope, above a minimum concentration (MHC), is expected to form organized micro-assemblies with distinct hydrophobic regions where the

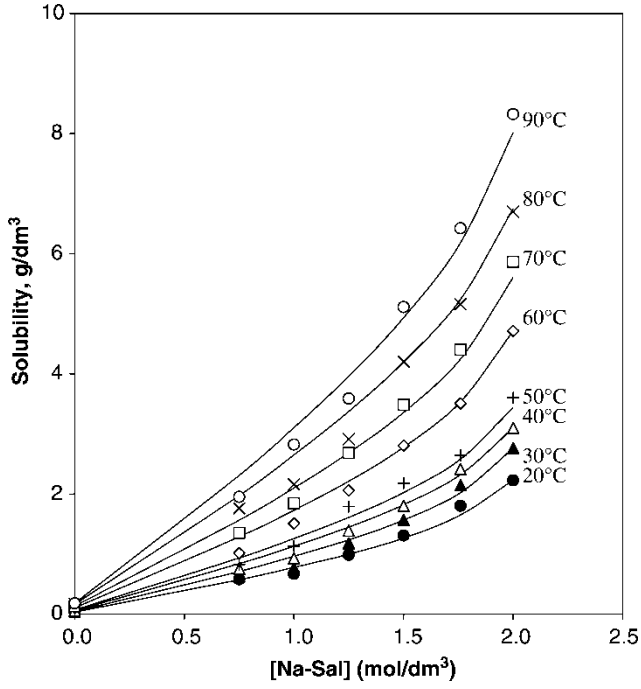

Figure 2. Effect of temperature on solubility of andrographolide in aqueous Na-Sal solutions.

solute can be solubilized. The solubilization of the solute is influenced therefore by the hydrophobic part i.e. the chain length of an alkyl group of the hydrotrope. The solubilization of an organic solute possibly occurs by intercalation between the hydrotrope molecules or by co-aggregation with the hydrotrope aggregates (15–18). The formation of a stable co-aggregate depends on the molecular geometry and the functional group(s) of the solute. A modified chemical association model (19, 20) of hydrotropic solubilization considers stepwise aggregation of hydrotrope molecules and solubilization of the solute *via* its co-aggregation subsequently with the hydrotropic aggregates. The self-aggregation of a hydrotrope which is favored by the hydrophobic effect is mainly governed by its hydrocarbon structure and opposed by the electrostatic repulsion between the charged head groups giving rise to an optimum aggregation number (m). The model relates aggregation constant (K_n) of formation of an n-mer to the dimerization constant of the hydrotrope (K_2) as $K_n = mK_2/n$ where 'm' is an optimum size of the aggregate. From this relation the association constant for the formation of an n-mer increases if the aggregation number is smaller than the optimum size otherwise it decreases, i.e. if $m > n$ then $K_n > K_2$ while if $m < n$ then $K_n < K_2$. The total concentration of the hydrotrope (C_s), and monomer

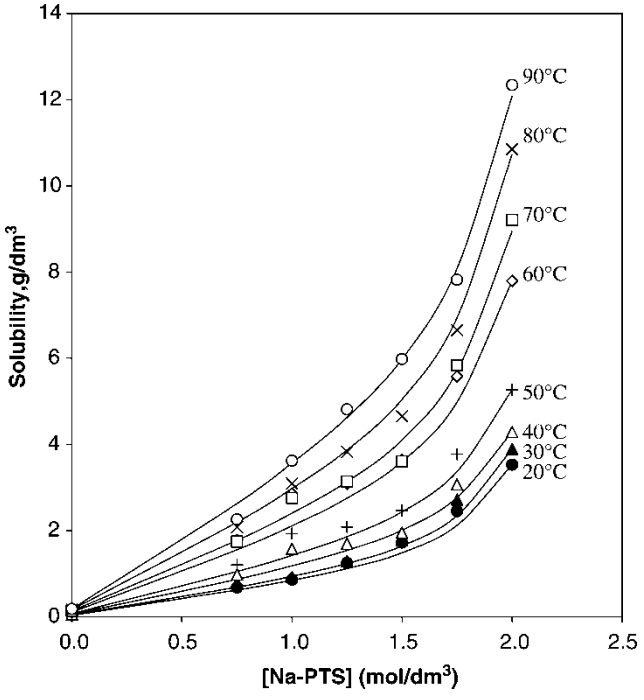

Figure 3. Effect of temperature on solubility of andrographolide in aqueous Na-PTS solutions.

concentration $[H_1]$ can be related by equation (1) (20)

$$C_s = 2\frac{[H_1]}{m}\left[\left(\frac{m}{2} - 1\right) + 2\exp(K_2 m[H_1])\right] \tag{1}$$

A solute molecule residing between the hydrotrope molecules can reduce the electrostatic repulsion between the charged groups of the neighboring hydrotropes, effectively tightening the aggregate structure and providing a geometrical constraint to the incorporation of more solute molecules into the same aggregate. The total amount of the solute associated with the hydrotrope aggregates, under the assumption that hydrotrope aggregate-solute association constant decreases with increase in solute concentration in an aggregate, is given by (20)

$$S_T = 2\left(\frac{K_s}{K_2}\right)\frac{[S_1]}{m^2}[\exp(mK_2[H_1]) - (1 + K_2[H_1])] \tag{2}$$

where the constant K_s characterizes the interaction between a solute-free hydrotrope aggregate and the first solute molecule and is taken independent

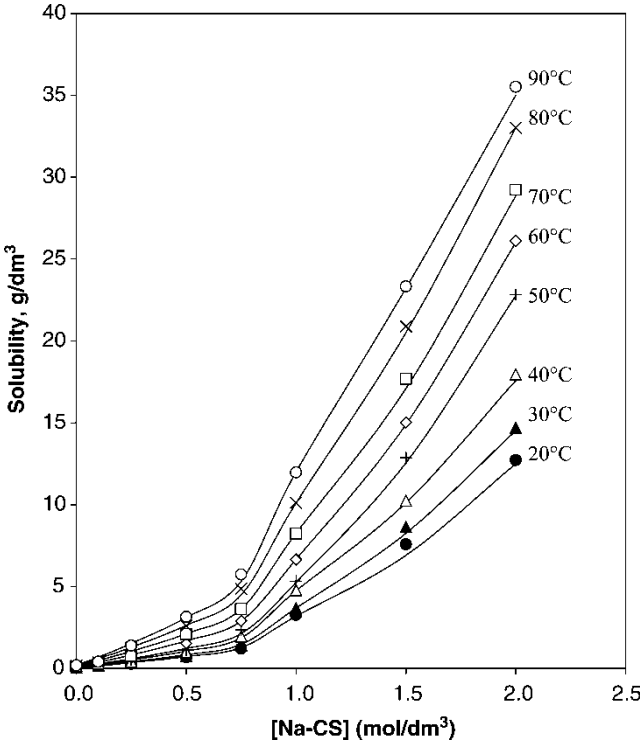

Figure 4. Effect of temperature on solubility of andrographolide in aqueous Na-CS solutions.

of the aggregation number of the hydrotrope aggregate. A higher value of K_s signifies stronger interaction of the solute with the hydrotrope aggregate.

The solubility values of andrographolide, were fitted into the modified chemical association model using non-linear regression to estimate the parameters, K_s, K_2 and m. Table 1 shows that andrographolide's interaction with Na-CS is the strongest followed by that with Na-PTS and Na-Sal as shown by the K_s values with the respective hydrotropes. The K_s for Na-CS is 166 dm^3/mol while 91 dm^3/mol and 81 dm^3/mol are the values for Na-PTS and Na-Sal, respectively. The hydrotrope self-association constant (K_2), on the other hand, shows comparatively very low values indicating not so strong aggregation tendency among these amphiphilic molecules themselves (Table 1). Na-CS shows an optimum aggregation number (m) of 21 while Na-PTS and Na-Sal show m equal to 19 and 18, respectively. The optimum aggregation numbers obtained for Na-CS, Na-PTS and Na-Sal are comparable to that obtained for Na-*n*-butyl benzene sulfonate (Na-NBBS) by small angle neutron scattering experiments (21). The reported value for m for Na-NBBS is about 30 (21).

Table 1. Hydrotrope-solute (K_s), hydrotrope-hydrotrope (K_2) interaction constants and optimum aggregation number of hydrotrope (m)

Hydrotrope	Temperature (°C)	K_s dm^3/mol	K_2 dm^3/mol	m
Na-CS	20	166.3	0.145	~21
	30	154.8	0.145	
	40	148.9	0.145	
	50	138.9	0.144	
	60	130.3	0.141	
	70	128.5	0.141	
	80	129.1	0.140	
	90	139.9	0.140	
Na-PTS	20	91.6	0.081	~19
	30	81.3	0.081	
	40	77.6	0.078	
	50	76.2	0.078	
	60	76.2	0.078	
	70	68.2	0.078	
	80	68.0	0.078	
	90	75.0	0.076	
Na-Sal	20	81.3	0.076	~18
	30	81.0	0.076	
	40	72.0	0.074	
	50	68.9	0.074	
	60	60.0	0.074	
	70	57.5	0.073	
	80	58.8	0.070	
	90	63.0	0.071	

Different hydrotropes having different characteristic MHCs show a varying extent of solubilization of andrographolide with their concentration (15–19). The solubility of andrographolide also shows that alkyl chain length and functional group attached to the aromatic ring play a role in the solubilization. The solubility of andrographolide in aqueous Na-CS solutions was enhanced to the extent of 330 times its solubility in pure water. In water, the solubility of andrographolide is only 0.035 g/dm^3 at 20°C while the same in aqueous Na-CS solutions is 15 g/dm^3 and 3 g/dm^3 in 2.0 mol/dm^3 Na-Sal solutions. For NaCS solutions, the increase in solubility of andrographolide was more significant beyond 1.0 mol/dm^3 hydrotrope concentration. The andrographolide solubility increased to 50 times its water solubility in 1.0 mol/dm^3 Na-CS solutions and 200 times in 2.0 mol/dm^3 Na-CS solutions. The solubility of andrographolide in ethanol (95%) is 10.2 gm/dm^3 at 30°C, which is lower in comparison to that in aqueous Na-CS solutions under similar conditions.

The temperature effect on the solubility of andrographolide can be due to the modified aggregate structures of hydrotropes at higher temperatures. However, in the absence of any information about the aggregation structure at higher temperatures it is difficult to explain the temperature effect.

The hydrotrope's self-association constant (K_2) decreases with increase in temperature indicating poorer association among hydrotrope molecules at higher temperatures. The aggregation of a hydrotrope is driven by weak hydrophobic effect because of its amphiphilic structure. But the hydrophobic part of the hydrotrope is smaller and thus lower values of self-association constant are not surprising. A slightly higher value of K_2 of Na-CS as compared to the other hydrotropes indicates its better ability to aggregate and, therefore, also to dissolve organic solutes better. Even though the solubility of andrographolide is higher at higher temperatures, K_s decreased with increase in temperature as the relative change in solubility with respect to the water solubility decreased at higher temperatures at a given hydrotrope concentration. The solubility of andrographolide in aqueous Na-CS solutions was affected the most at higher temperatures as compared to that with other hydrotrope solutions. Increasing temperature from 20°C to 90°C, the andrographolide solubility increased 4 to 5 times in aqueous Na-CS solutions below 1.0 mol/dm^3 hydrotrope concentration. At still higher Na-CS concentrations, the temperature effect on the solubility of andrographolide reduced significantly. Since the solubility of andrographolide is higher in the Na-CS solutions compared to that with other hydrotropes as well as in ethanol (95%), further extraction experiments were conducted with aqueous Na-CS solutions.

Extraction Studies with Hydrotrope Solution

The solid suspension density of the leaves in the extraction process was maintained 5% (w/v) in the aqueous hydrotrope solutions as well as in the case of organic solvents. At higher solid loadings of the raw material, the suspension became viscous and thick due to absorption of hydrotrope solutions into the solid particles which also made the sampling as well as the filtration of the slurry difficult. The extraction data obtained for andrographolide were fitted in a first-order kinetic equation (Eqn. 3) to estimate an extraction rate constant (k), the reciprocal of which represents the characteristic time of the extraction i.e. a higher value of 'k' corresponds to a higher rate of extraction.

$$\% \text{ extraction} = b(1 - e^{-kt}) \tag{3}$$

where b is maximum extraction achieved at the specified conditions.

Effect of Particle Size on Extraction

Figure 5 shows the effect of particle size on the andrographolide extraction using 1.0 mol/dm^3 aqueous NaCS solutions. Smaller size particles, in the

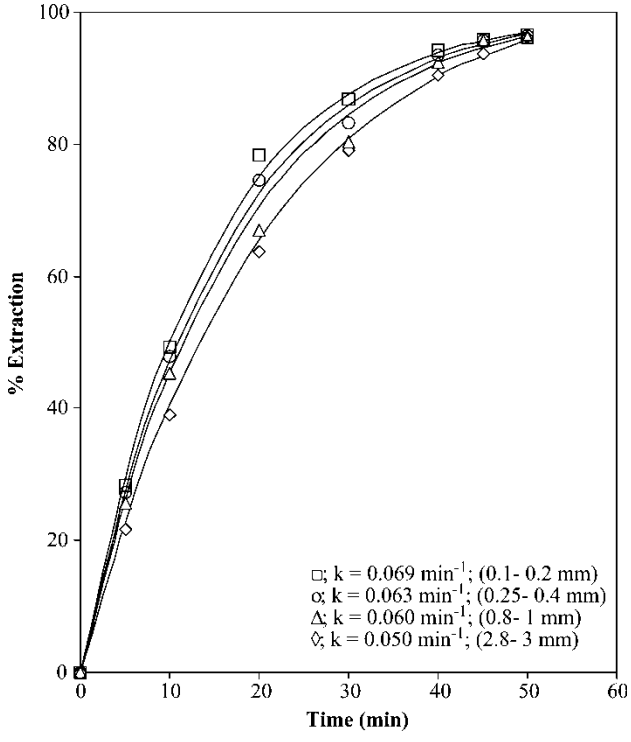

Figure 5. Effect of particle size on the rate of extraction of andrographolide using 1.0 mol/dm^3 aqueous Na-CS solutions at 30°C [solid loading 5% (w/v), time: 60 min].

range 0.25–0.4 mm and 0.1–0.2 mm, absorbed a significant amount of aqueous solutions and formed a thick paste. This made the processing of the suspension difficult and extraction with larger size particles became necessary from the operational point of view. The rate of extraction depends on how easily the hydrotrope solution penetrates the solid matrix and enables the solubilization of andrographolide. The extraction data showed a first order behavior with respect to the andrographolide concentration in the aqueous hydrotropic solutions. The extraction rate constant showed a higher value (0.060 min^{-1}) as compared to those reported for curcuminoids (0.0101 min^{-1}) and dioscin (0.0133 min^{-1}) (6, 9) extractions from respective rhizomes. In the latter cases a larger biomatrix had to be penetrated before the hydrotrope solution could access the active components. The extraction rate constant increases with reduction in the particle size indicating the major resistance to mass transfer to be within the particles. Below 0.8–1 mm, the particle size of the raw material had no significant effect on the andrographolide extraction rates.

Extraction with Different Solvents

Figure 6 shows extraction of andrographolide with different organic solvents such as ethanol (95%), methanol, acetone, dichloromethane (DCM) and chloroform. The extraction rate of andrographolide with acetone was the highest amongst the organic solvents but still slower than rates obtained using Na-CS solutions. The extraction rate also showed a first order dependence with respect to the andrographolide concentration in all the solvents. Figure 7 shows purity of andrographolide recovered from different solvents and also from hydrotrope solutions. The highest purity of 94% with maximum extraction of 96% was obtained with Na-CS solutions. Among the organic solvents, DCM gave the maximum purity of 31% of the extract with ~50% of andrographolide extraction compared to the other solvents. Wongkittipong et al. (6) have reported 75% extraction of andrographolide from the raw material comprising of leaves and stems using ethanol (80%) with the maximum solid loading of 2% whereas we have observed 96%

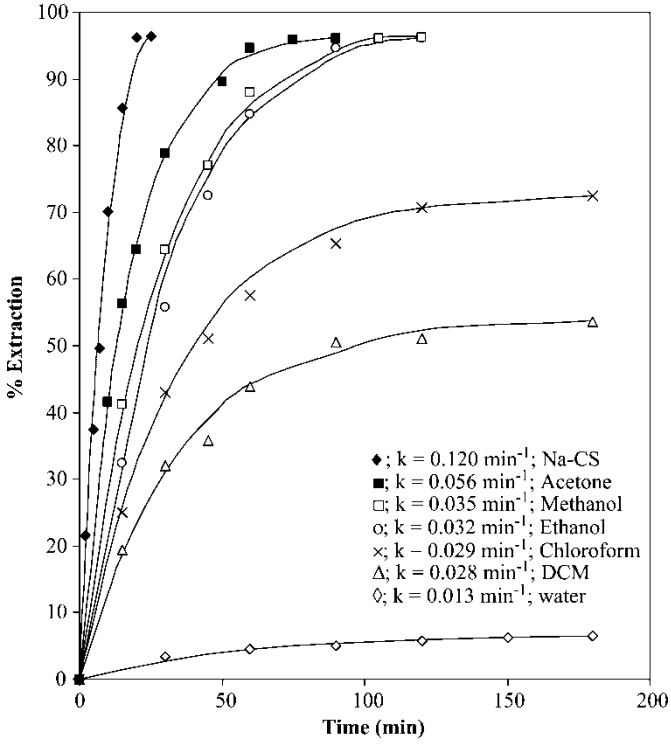

Figure 6. Comparison of different solvent used for andrographolide extraction with aqueous Na-CS solutions [solid loading 5% (w/v), 0.8–1 mm size particles, time: 180 min].

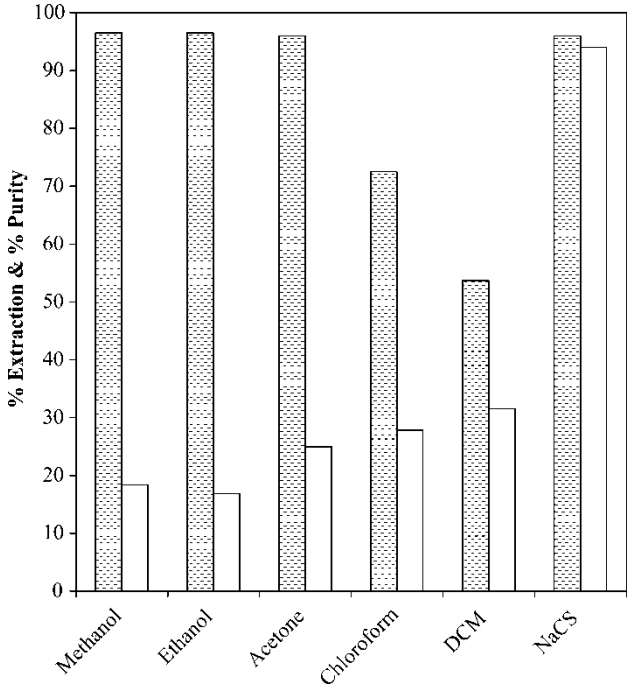

Figure 7. Solvent effect on the percentage extraction and purity of andrographolide recovered [solid loading 5% (w/v), 0.8–1 mm size particles, time: 180 min]. % Extraction ▭, % purity □.

extraction with ethanol (95%) at the maximum solid loading of 5% of the leaves.

Extraction Studies with Different Hydrotropic Solutions

Figure 8 shows a comparison of the andrographolide extractions using different hydrotrope solutions at 30°C. The extraction studies were conducted with hydrotrope solutions of 1.0 mol/dm^3 concentration each and with 0.8–1 mm size particles at 5% solid loading (w/v). Aqueous Na-CS solutions could extract 96% of andrographolide in 50 min while Na-PTS and Na-Sal could extract ~90% and 63%, respectively, in 3 hr. The higher solubility of andrographolide in the Na-CS solutions as compared to that in the Na-PTS and Na-Sal solutions should be responsible for the higher rates of extraction of andrographolide by Na-CS.

Figure 9 shows extraction of andrographolide with aqueous NaCS solutions of different concentrations in the range 0.25 to 2.0 mol/dm^3, with 0.8–1 mm size powdered leaves and also that with plain water. Only 6.5% of total andrographolide present in the leaves was extracted with pure

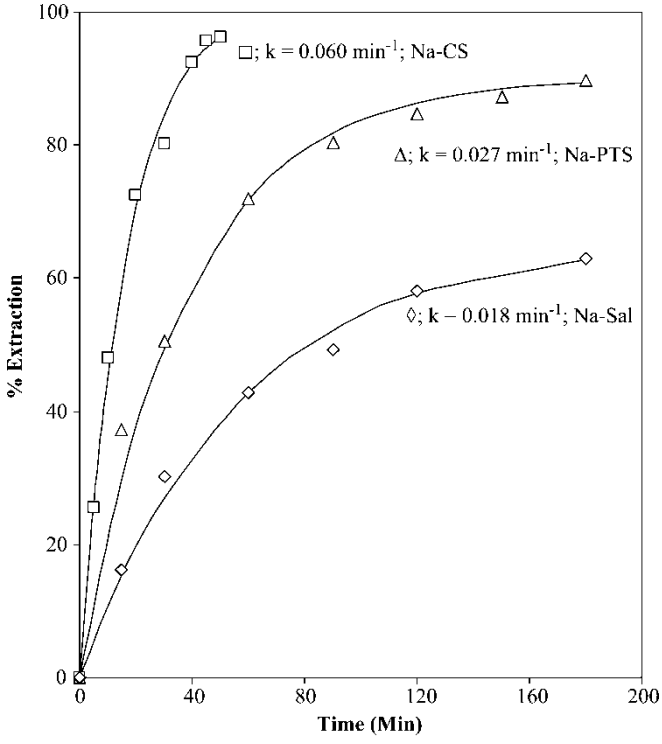

Figure 8. Comparisons of andrographolide extraction with different hydrotropes at 30°C [solid loading 5% (w/v), 0.8–1 mm size particles, time: 180 min].

water. Figure 9 shows a significant increase in the andrographolide extraction at higher hydrotrope concentrations. Na-CS solutions (0.25 mol/dm^3) extracted 35% of andrographolide in 3 hr at 30°C while 2.0 mol/dm^3 Na-CS solutions extracted 96% of andrographolide within 20 min. The extraction was limited to 35% with 0.25 mol/dm^3 hydrotrope concentration as the solubility of andrographolide is low at this hydrotrope concentration. A greater extent of permeabilization of the cell wall as well as higher solubility of andrographolide lead to higher extraction rates in the Na-CS solutions at higher hydrotrope concentrations. Figure 9 shows the hydrotrope concentration dependence of the extraction rates. The extraction rate constant increased from 0.014 to 0.12 min^{-1} with the increase in the hydrotrope concentration. The k's at different hydrotrope concentrations indicate that the extraction of andrographolide increases almost linearly with the hydrotrope concentration at a given temperature.

Figure 10 shows the effect of temperature on the rates of andrographolide extraction in the range 30°C to 90°C using 0.25 mol/dm^3 Na-CS hydrotrope solutions. The extraction of the active material increased with the increase in temperature. These results need to be interpreted in terms of plant cell

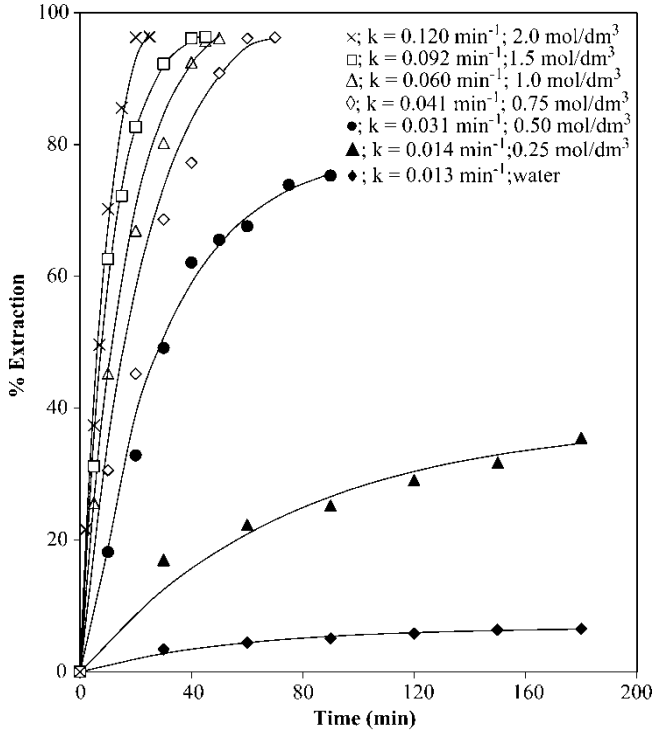

Figure 9. Effect of aqueous Na-CS concentration on the percentage extraction of andrographolide at 30°C [Na-CS: 0–2.0 mol/dm^3, solid loading: 5% (w/v), 0.8–1 mm size particles, time: 180 min].

structure and the effects of hydrotrope and temperature on the same. Epidermis is a permanent tissue forming the outermost layer of the plant structure and normally is one cell thick (2, 3). The outer walls of the epidermal cells are often thick and covered with a fatty substance called cutin. The layer with cutin is called cuticle. This nature of the leaves offers a resistance to penetration by hydrotrope molecules. Hydrotropes are thought to have disruptive effects on the cell membranes in addition to hydrolytic effect on the cellulosic structures of plant cells (10). At higher temperatures, an increased rupture of the cellular structure is possible by hydrolysis of cellulose followed by increased solubilization of solute into the hydrotropic solutions. Due to the breakdown as well as solubilization of the cellulose polymers from the cell wall, the contribution of cellulose towards the firmness of the cell wall could be reduced enabling faster rates of extraction of active materials.

At higher temperatures, the extraction rate constant expectedly increased, i.e from 0.014 per min at 30°C to 0.19 per min at 90°C indicating higher

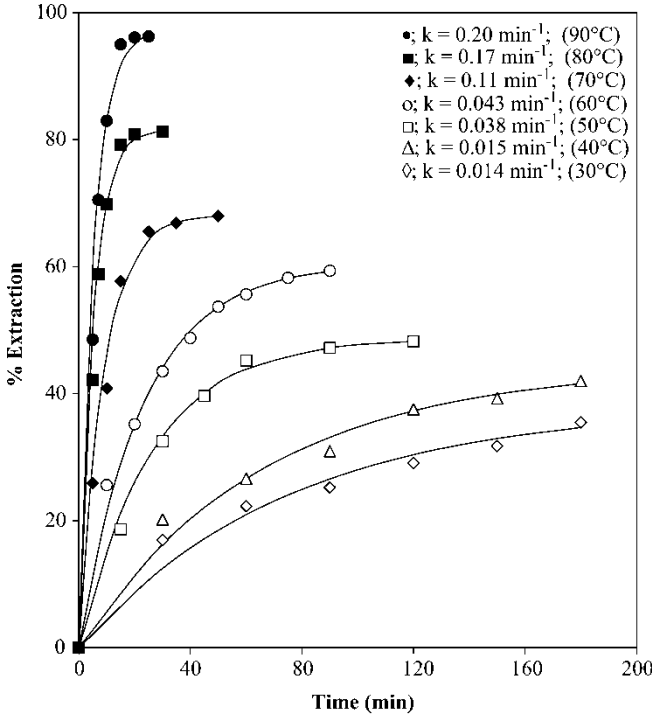

Figure 10. Effect of temperature on the extraction of andrographolide using aqueous Na-CS solutions [Na-CS conc: 0.25 mol/dm^3, solid loading: 5% (w/v), 0.8–1 mm size particles, time: 180 min].

extraction rates. At 90°C, 96% extraction of andrographolide was completed within 15 min. It was expected according to solubility values that 80% of the extracted andrographolide would precipitate out on cooling the hydrotropic solutions from 90°C to 20°C. However, in actual only ∼5% of the total extracted andrographolide precipitated as a solid product from the hydrotropic solutions on cooling to 20°C indicating a slow release of the material from the hydrotrope aggregates.

A mass transfer model of extraction of active material from solid matrix as reported in literature (6) was used to mathematically characterize the extraction process of andrographolide. A second order finite difference method was used to discretize the relevant differential equations to solve by the Crank-Nicolson method. The diffusion coefficient of andrographolide within the solid particles was estimated by the regression analysis with the experimental data. Figure 11 shows that experimental values are well represented by the given model with a plate shape factor. Diffusion coefficients of andrographolide for different solvents as well as at different hydrotrope concentrations are reported in Table 2. The diffusion coefficient varies in

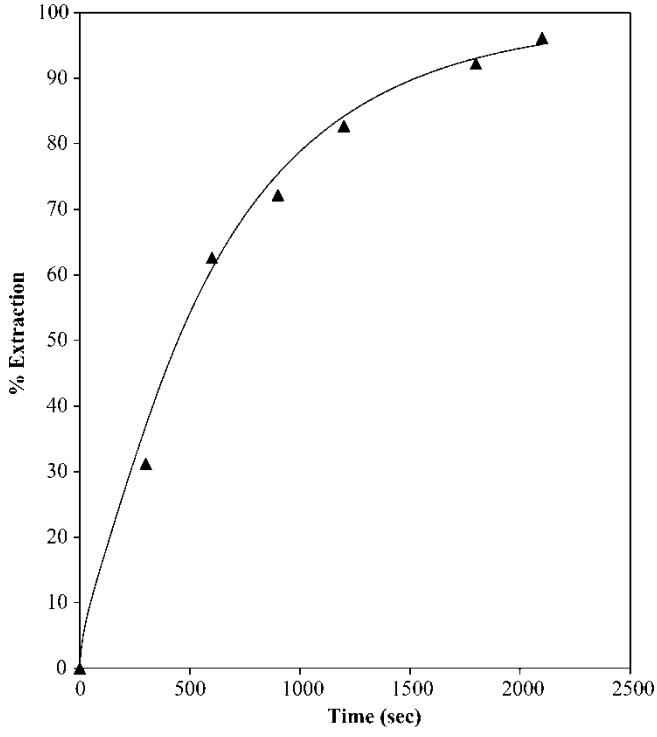

Figure 11. Experimental (symbols) and predicted (line) values by mass transfer model for the recovery of andrographolide [solid loading: 5% (w/v), 0.8–1 mm size particles, time: 180 min].

the range 2.58×10^{-15} to $6.94 \times 10^{-15}\,m^2/sec$ for different solvents which also shows very slow mass transfer rates inside and from the particles. The diffusion coefficient increased linearly with the increase in hydrotrope concentration indicating a greater extent of penetration of hydrotrope molecules into

Table 2. Diffusion coefficient of andrographolide in the solid phase in presence of different solvents and hydrotrope concentrations at various temperatures

Na-CS (mol/dm^3) at 30°C	$D \times 10^{-15}$ ($m^2\,s^{-1}$)	Na-CS (0.25 M) Temp (°C)	$D \times 10^{-15}$ ($m^2\,s^{-1}$)	Solvent 30°C	$D \times 10^{-15}$ ($m^2\,s^{-1}$)
0.25	2.76	40	3.85	Water	2.58
0.50	4.36	50	5.60	DCM	3.66
0.75	6.92	60	6.77	Chloroform	4.32
1.0	9.34	70	13.5	Ethanol	6.26
1.5	14.7	80	21.3	Methanol	6.53
2.0	24.8	90	27.5	Acetone	6.94

the cell structure. This can lead to cell structure lysis to a greater extent and therefore, to the increased andrographolide extraction rates. Table 2 also shows that diffusion coefficient of andrographolide in the solid structure increases with the increase in temperature, thereby indicating higher mass transfer rates of andrographolide at higher temperatures.

Recovery of Andrographolide

The organic solutes from hydrotrope solutions are usually recovered by dilution with water. But this method requires a large amount of water to bring the hydrotrope concentration below its MHC as well as an energy intensive water evaporation process for recycle of the hydrotrope. In addition, the recovery of andrographolide by dilution was extremely slow and incomplete. We, therefore, propose recovery of andrographolide from the hydrotropic solutions by partitioning with an organic solvent. It is possible to recover andrographolide from the aqueous hydrotropic solutions by a secondary solvent extraction process. Using chloroform (or DCM) in equal volume ratio, 70% of andrographolide from hydrotrope solutions was extracted at 30°C in a single stage. The partition coefficient of andrographolide was 2.35 for chloroform and 2.44 for DCM at 30°C, respectively. In two cross-current stages ~95% of dissolved andrographolide was extracted from the aqueous hydrotropic phase into the organic phase. The purity of andrographolide so recovered in the organic solvent was 94% and 92% for $CHCl_3$ and DCM extractions, respectively. The partitioning with $CHCl_3$ of the hydrotrope extract solution obtained at 40°C and 50°C, gave 75% and 77% recovery of andrographolide in a single stage extraction process but the purities of the recovered product reduced slightly to 91% and 90%, respectively. The extraction and partitioning at lower temperatures gave a higher purity product because the aqueous hydrotropic solutions retained most of the impurities.

A better approach to obtain andrographolide is thus to conduct the extraction of andrographolide from *Andrographis paniculata* leaves at higher temperatures using hydrotrope solutions and partitioning of the same extract solutions with organic solvent to recover the product into the organic phase. The hydrotrope solution as well as the organic solvent, after crystallizing andrographolide, can be recycled to the respective steps.

Recyclability of Hydrotrope Solutions

The reusability of 0.25 mol/dm^3 aqueous Na-CS solutions was verified after each extraction with 5% (w/v) solid loading of average 0.8–1 mm particles. The hydrotrope solution was recycled for five times and each time fresh leaves were charged for the extraction run. The degree of extraction of

andrographolide remained almost constant (96–93%) up to four runs and decreased only to 90% in the fifth run. At each extraction stage, there was ~3% (w/w) loss of the hydrotrope solution with the solid cake but it was completely recovered by simple water washing. Extraction of some saponifiable materials such as saponins also made the solution to froth and foam. This frothing of the solution increased with the increase in number of recycles and a treatment with activated carbon is necessary to deal with the foam by removing the saponins and coloured impurities from the hydrotrope extract solutions (10, 22).

CONCLUSION

Andrographolide can be extracted using aqueous hydrotrope solutions from *Andrographis paniculata* leaves. Sodium cumene sulfonate gave the best extraction rates and efficiency in cell permeabilization and solubilization of andrographolide. The solubility of andrographolide increased by two orders of magnitude in the presence of hydrotropes in the aqueous solutions. Extraction of andrographolide using hydrotropic solutions followed by partitioning with organic solvent gave andrographolide of better purity. The extraction of andrographolide using hydrotropes is completed within 20 min which is comparable to other conventional processes using organic solvent. The kinetics of extraction predicted by the mass transfer model shows the intraparticle diffusional process to be the rate controlling process.

APPENDIX I

Andrographolide

The infrared (IR) spectrum showed the presence of hydroxyl (3396.8 cm^{-1}), α, β-unsaturated-γ-lactone (1726.6 cm^{-1} 1674.7 cm^{-1}) and exo-methylene (907.0 cm^{-1}) groups. *^{1}H-NMR* (DMSO) 300 *Mhz*, Chemical shifts (δ), 0.66 (3H, s, CH_3-20); 1.1 (3H, s, CH_3-18); 1.9 (signal overlapped) (H-9); 2.45 (br t, J = 7.5 H_z, H-11); 3.22–3.35 (signal overlapped) (2H, br m, H-19$_A$, H-3); 3.86 (1H, d, J = 9.6 H_z, H-19$_B$), 4.30 (1H, dd, J_1 = 10.2 Hz J_2 = 2.4 H_z, H-15$_A$), 4.42 (1H, dd, J_1 = 9.9 H_z J_2 = 6.0 H_z, H-15$_B$); 4.90 (br s, H-17); 5.32 (br m, H-14); 6.7 (1H, td, J_1 = 6.3 H_z, J_2 = 0.9 H_z, H-12).

NOTATIONS

C_s	total concentration of the hydrotrope (moles/dm^3)
$[H_1]$	monomer hydrotrope concentration (moles/dm^3)
K_2	hydrotrope- hydrotrope interaction constant (dm^3/mol)
K_s	solute-hydrotrope interaction constant (dm^3/mol)

m	optimum aggregation number
S_1	solubility of andrographolide in water (g/dm^3)
S_T	increased solubility due to hydrotrope solution (g/dm^3)
t	time
k	extraction rate constant
b	maximum extraction achieved at the specified conditions.

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