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Hydrotropic Extraction of Curcuminoids from Turmeric

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ABSTRACT

A novel hydrotropy-based extraction method for selective extraction of curcuminoids from *Curcuma longa* (turmeric) was investigated. The degree of extraction was dependent upon the effect of a hydrotrope on the cellular structure and hydrotrope–curcuminoids interactions. Hydrotropes directly affect the cell structure, making curcuminoids more accessible either by dissolution of the cell membrane/wall constituents or by disorganizing cell wall. Sodium cumene sulfonate (Na-CuS) was found to be an efficient hydrotrope for the extraction of curcuminoids. A significantly strong interaction with sodium n-butyl benzene sulfonate (Na-NBBS) of curcuminoids was undesirable in the recovery of curcuminoids from the extract phase, although Na-NBBS was extremely efficient in extracting curcuminoids. The two-step process, hydrotropic solubilization followed by dilution using water with or without pH adjustment, gave good yields of curcuminoids with high purity.

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Key Words: Curcuminoids; *Curcuma longa*; Turmeric; Hydrotropy; Sodium cumene sulfonate; Sodium butyl benzene sulfonate.

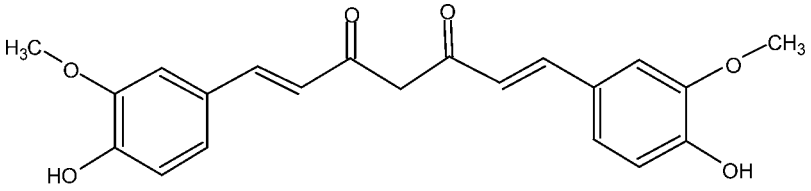
INTRODUCTION

The rhizomes of the plant *Curcuma longa* L., commonly known as turmeric, have a traditionally important role as natural coloring agent in food, cosmetics, and textiles. Curcumin, main coloring substance in *Curcuma longa*, and two related compounds, demethoxycurcumin and bis-demethoxycurcumin, are together known as curcuminoids (Fig. 1). These are proven anticoagulative, antibacterial, anti-inflammatory, antifungal, antiparasitic, and antimutagenic agents.[1,2] Curcuminoids also show a potent anticancer activity[3] and are modest inhibitors of HIV-1 and HIV-2 proteases.[4]

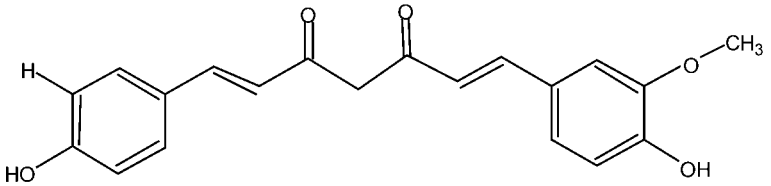
Considering the potential of curcuminoids, attempts were made by several researchers in the past to isolate curcuminoids from turmeric rhizomes by solvent extraction using organic solvents.[5–10] Defatting of raw material prior to extraction and purification of curcuminoids after the extraction was, however, necessary. Curcuminoids are recovered from the organic solvent extract by solvent evaporation, but usually residual solvent remaining in the extract is very difficult to remove. The use of large volumes of volatile and inflammable organic solvents also makes the extraction process labor intensive, hazardous, and environmentally unfriendly.

In the recent years, supercritical fluid-based extraction has gained commercial importance as an efficient method of extraction for natural products. It has been investigated for the extraction of essential oils from *Curcuma longa*.[11,12] Recently, Baumann et al.[13] have claimed efficient extraction of curcuminoids using supercritical CO_2 modified by 10% ethanol. Although supercritical fluid extraction is known to be a clean technology giving acceptable yields and purity, its major disadvantage lies in its high operating pressures. The scaleup problems could also be severe when the extraction is to be done at large scales.

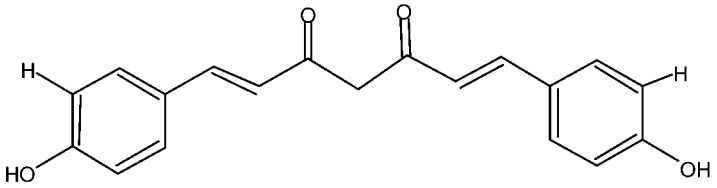
Stransky[14] has patented a process for extraction of curcuminoids using vegetable oil-based alkaline soap solutions. The extract could be directly used as food coloring agent or the pigments could be precipitated by acidification. As curcuminoids are phenolic compounds, extraction with aqueous alkaline solutions can be employed. However, curcuminoids are unstable in alkaline conditions and the degradation rate increases rapidly at pH 7.45, giving a maximum at pH 10.2, where the half-life of curcumin is reported to be half an



Curcumin



de-Methoxy curcumin



Bis de-methoxy curcumin

Figure 1. Structure of curcuminoids.

hour.[15,16] Though these processes are aqueous solution based, they are not very efficient due to degradation of curcuminoids in the extraction process.

In recent years we have investigated the phenomenon of hydrotropy for a number of potential applications in process industry.[17–19] In this paper, we report a new and more efficient hydrotropy-based process for extraction of curcuminoids from turmeric rhizomes. The process is free from organic solvents, and the capacity of hydrotrope solutions to solubilize curcuminoids surpasses the capacity of organic solvents with concomitant faster rates of extraction.

Hydrotropy is the phenomenon of increasing solubility of water-insoluble or sparingly water-soluble organic compounds in aqueous solutions in the presence of hydrotropes. Hydrotropes themselves are highly water-soluble organic salts. The increase in solubility of an organic substance is a strong function of the hydrotrope concentration and depends not only on the nature of hydrotrope but also on the nature of solute. The manipulation of hydrotrope concentration gives a convenient recovery technique of solute, i.e., a mere dilution of hydrotropic solution by water precipitates the solute.

Amphiphilic organic substances with a short linear or branched alkyl chain or an aromatic ring with a short alkyl chain, attached to a strongly polar/ionic group, can be used as hydrotrope. Hydrotropes show amphiphilic character as surfactants but have poorly hydrophobic characteristics compared to conventional surfactants. Hydrotropy is believed to be a collective molecular phenomenon, and self-aggregates of hydrotrope molecules are implicated to be the functional species for hydrotropy.[20] In hydrotrope solutions, the solubility enhancement is appreciable only above a certain minimum hydrotrope concentration (MHC) in a manner analogous to critical micellar concentration (CMC) of a surfactant, but unlike surfactants, where a sharp break is observed in the solubilization characteristics at CMC, the solubility curves in hydrotrope solutions show a gradual variation above MHC over a wide range of concentration. The micelles in surfactant solutions provide the pseudo-phase for solubilization of organic hydrophobic compound either on the surface of the micelles or in the interior of the micelles.[20] The hydrotrope assemblies, which are supposed to provide a micro-environment similar to that an organic solute experiences while associated with a surfactant micelle, cannot be as compact as micelles as evident from the molecular structures of commonly used hydrotropes.

Alkali metal salts of benzoic and substituted benzoic acids, benzene sulfonic acid and its many derivatives, naphthoic acid, and various hydroaromatic acids are typical hydrotropes. The hydrocarbon part of these hydrotropes is much smaller in comparison to that of a surfactant, and it is difficult to envisage micelle-like structures of their aggregates. The aromatic hydrotropes are expected to form large stack-like but somewhat open structures in contrast to the compact assemblies formed by surfactants.[21] The nonpolar molecules would enter the hydrophobic layers of these assemblies, intercalating themselves between the layering molecules, and in turn can stabilize the layered structures producing a cooperative solubilizing isotherm. This way the selectivity in solubilization is observed with a solute that intercalates well and stabilizes the layered structure more, whereas poorly interacting bulky hydrophobic compounds are not solubilized to the same

efficiency. While there are several points of resemblance between hydrotrope molecules and surfactant, e.g., aggregation, solubilization of hydrophobic compounds, surface activity, etc., there are also significant differences, i.e., not all hydrophobes are solubilized by hydrotropes and concentration-dependent variation of properties of hydrotropes is not as high as that shown by surfactant, i.e., very high MHC compared to CMC.

The major research in earlier years on hydrotropy was concentrated on applications of hydrotropes in drug and detergent formulations, while their applications in chemical engineering field received impetus only in the recent years.[17–19,22–24] Hydrotropes were successfully used in enhancing the rates of heterogeneous chemical reactions such as the oxidation of cyclodecanone, ester hydrolysis, Canizarro and cross-Canizarro reactions, Hantzsch pyridine synthesis, etc.[17,22–24] Our group in recent years showed that hydrotropes could also be successfully utilized in the extractive separations and extractive distillations of close-boiling substances.[17–19] The use of hydrotropes in these applications is attractive because of the high selectivity in separations and easy recovery techniques.

The question that we asked here is whether hydrotropy can be used for extraction of water-insoluble organic active ingredients from complex biomatrices. The high solubilization capacity and selectivity in solubilization by hydrotropes should be useful for the natural products extraction. As an example we have studied curcuminoids from turmeric because of two reasons. First, curcuminoids are commercially important, and second, being phenolic compounds because of their molecular structures, they should have good solubility in hydrotrope solutions.

The use of aqueous solutions avoids potential hazards associated with the organic solvents commonly used for such extraction. At the same time, the question of residual solvent traces remaining with the final product does not arise. Since hydrotropes are highly water soluble, traces of hydrotrope molecules adhering to the final solid precipitate, if any, can be washed off easily with water. Moreover, the diluted hydrotrope solution, offer recovery of solute, can be concentrated by evaporation, as hydrotropes are thermally stable nonvolatile organic salts, for recycle into the extraction stage. This recycle also reduces the cost of extraction.

Turmeric rhizomes consist of various layers of different cells and curcuminoids and are mainly present in inner cell layers, i.e., cortex and central cylinder in oleoresin cells.[25] Dead cork cells, which are impermeable to water, cover the cortex composed of inner and outer cellulose layers and a median suberin lamella, and that makes the access to the curcuminoids difficult for the penetrating medium.

In order to dissolve curcuminoids the hydrotrope solutions should penetrate the cellulosic layers of each cell to access the curcuminoids. We expect either hydrotrope solution to partly dissolve the cell wall/membrane constituents and/or at least destabilize the cell wall structure during the process. Hydrotropes show a property of destabilization of lamellar liquid crystalline phases of a conventional surfactant in aqueous solutions.[26] Surfactant solutions form different aggregating solutions in aqueous solutions. At fairly high concentrations, surfactants, because of their own poor solubility in water, form lamellar ligand crystalline structures in the solutions. These structures are reminiscent of the cell membranes, where double phospholipid layers provide the structural integrity to the cells. The nature of these amphiphilic molecules also imparts the well-known characteristic of semi-permeability to the cell membranes. The lamellar liquid crystalline structures of surfactants in aqueous solutions have been extensively studied because of their similarity with the natural membrane structure. However, on increasing concentration of surfactant in the presence of hydrotrope, isotropic nature of the solutions was maintained over a wide range of concentration.[26] The hydrotrope seemed to be responsible for the disruption of the lamellar structures of surfactants. A hydrotrope, depending on its conformation and molecular structure, is expected in an analogous manner to disrupt the phospholipid bilayers present in the plant cell structures, and ultimately to break the cell wall or at least to make it more permeable to the solution. Consequent to this effect, higher rates of extraction are expected, and that indeed has been the observation in this work.

The destabilization of cellular structure should improve the accessibility to curcuminoids for the extracting hydrotropic solution. Also many hydrotropes, available in different forms, can show varied selectivity toward curcuminoids. This selective solubilization can be expected to substantially increase the purity of extracted product. Since curcuminoids are phenolic compounds, their solubilization by aromatic ring-based hydrotropes is expected to be more selective.

EXPERIMENTAL METHODS

Dry *Curcuma longa* rhizomes were obtained from M/s. Cancore Flavours and Extracts Ltd., Kochin, Kerala (India). The raw material was first pulverized, and the dry powder was separated by mechanical sieving into different mesh size batches, i.e., 6 # mesh, 22 # mesh, and 85 # mesh.

Sodium cumene sulfonate (Na-CuS) was purchased from M/s. Navdeep Chemicals, Mumbai, and purified by recrystallization. Sodium n-butyl

benzene sulfonate (Na-NBBS) was synthesized in laboratory by sulfonation of n-butyl benzene using H_2SO_4 (98% w/v), followed by neutralization with NaOH solution and finally by recrystallization. Sodium butyl mono glycol sulfate (Na-BMGS) was supplied by M/s. Hulls (Germany) as a 50% (w/v) solution in water. *p*-Toluene sulfonic acid (PTSA) (AR grade) was purchased from M/s. S. D. Fine Chemicals, Mumbai. Sodium salicylate (Na-Sal) (IP grade) was purchased from M/s. Khona Chemical Works, Mumbai. All other chemicals, like sulfuric acid (98%), hydrochloric acid, sodium hydroxide, etc., or solvents for analysis, like methanol, ethanol, chloroform, etc., (all AR grade) were purchased locally and were used as such.

The solubility of curcuminoids in different hydrotrope solutions was determined by addition of solid curcuminoids to hydrotrope solutions of varying concentrations, and the suspension was stirred using magnetic stirrer in a constant-temperature bath at 30°C for ten hours. The solubility was determined by analyzing the curcuminoids dissolved in hydrotrope solutions using UV–visible spectroscopy.

The extraction of curcuminoids from turmeric powder of selected particle size was conducted in a fully baffled cylindrical vessel of internal diameter 7 cm and height 9 cm, equipped with a four-blade turbine impeller of 2 cm diameter, by suspending the raw material in 100-ml hydrotrope solution maintained at an appropriate temperature. A few samples were collected from the solution during the extraction period and were analyzed for curcuminoids. After the extraction was complete, the extract solution was separated from the solid residue by filtration. The extract was then diluted using water (with or without pH adjustment as described later) below MHC of the hydrotrope to recover the curcuminoids, which precipitated out as fine crystalline solid product from the solution. A list of MHCs of hydrotropes is given in Table 1. The precipitated curcuminoids were then recovered by centrifugation or filtration. The solid was washed with water and a few samples were taken from the product for analysis of the curcuminoids content.

The solid suspension density in the extraction process was varied from 1% to 5% (w/v). It was observed that beyond the loading of 5% (w/v), the suspension became highly viscous due to swollen starchy grains of rhizome, and sampling as well as filtration of the extract became difficult. A loading of 5% (w/v) raw material was, therefore, used to study the effect of other parameters unless stated otherwise. The rate of extraction became independent of the speed of agitation beyond 1200 rpm, and thus the stirring rate was maintained in the range of 1200–1300 rpm. The extraction was generally carried out for 10 hours, unless mentioned otherwise to ensure equilibrium.

The total curcuminoids content in the raw material was determined separately by soxhlet extraction with acetone. The raw material was defatted

Table 1. The interaction constants of solute–hydrotrope and hydrotrope–hydrotrope interactions.

Hydrotrope	MHC (mol/dm^3)	k_s (dm^3/mol)	K_s (dm^3/mol)	K_2 (dm^3/mol)
Na-NBBS	0.1	6.983	122	0.148
Na-CuS	0.1	4.044	48	0.05
PTSA	0.38	2.538	30	0.068
Na-BMGS	0.7	2.904	45	0.059
Na-Sal	0.65	1.828	11	0.034

using petroleum ether (boiling point 40–60°C fraction) prior to extraction of curcuminoids for 16 hrs. The curcuminoids extraction was conducted for 10 hrs/day for 6 days using 30 gm of the defatted raw material and about 200 ml of acetone. Everyday the extract was removed and fresh solvent was added. The percent curcuminoids present in the raw material was determined to be 5.8% (w/w).

Thin sections of dry *Curcuma longa* rhizome were observed, before and after soaking in water and hydrotrope solutions separately, under microscope (Leica, Germany) of magnification 40 × and photographs were taken using an SLR camera.

ANALYTICAL METHODS

The curcuminoids were analyzed by high-performance thin layer chromatography (HPTLC) and UV–visible absorption spectroscopy. In the HPTLC method, the analysis was performed on 10 cm × 10 cm HPTLC silica gel 60 F_{254} plates from E. Merck (Germany).[27] The extracts were applied as 5-mm bands using Desaga Applicator AS 30. The separations were performed using chloroform–ethanol (95% wt)–glacial acetic acid (94:5:1, v/v) in a TLC chamber previously saturated for 15 minutes at room temperature. The plates were developed to a distance of 7 cm. The plates were then dried in air and scanned using Desaga Densitomer CD60 and Desaga Software CD60 at wavelength 423 nm. In the spectrophotometric method Hitachi (U-1100) UV–visible spectrophotometer was used with quartz cuvettes of 1 cm path length. The absorbance was recorded at wavelength 423 nm.

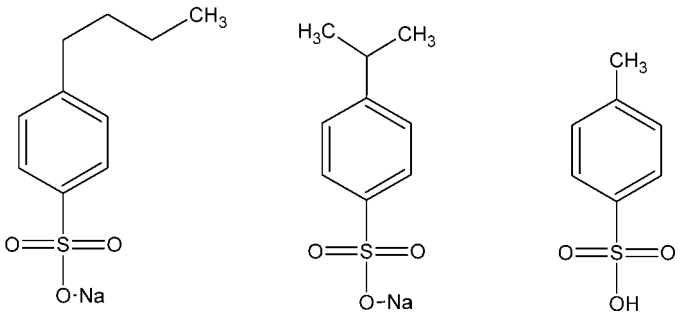
RESULTS AND DISCUSSION

Three different types of hydrotropes were selected for the extraction studies: alkyl benzene sulfonates, alkyl glycol sulfate, and aromatic carboxylate. The hydrotropes were selected on the basis of the expected efficiency of these hydrotrope classes. Since a phenyl group is considered to be equivalent to three or four carbons in a straight chain, it is possible that in the case of alkyl benzene sulfonates, a shorter alkyl chain would be sufficient. With the length of the alkyl chain the hydrophobic character of hydrotrope increases as does its solubilizing efficiency.[28,29]

Three hydrotropes from the alkyl benzene sulfonate class were used in the following studies with increasing chain length of the substituent alkyl group. The other hydrotropes, an alkyl glycol sulfate and aromatic carboxylate, were selected for comparison (Fig. 2). Initial studies of extraction were conducted first to find the efficacy of hydrotropes for extracting curcuminoids. Since curcuminoids are phenolic compounds, and an ability of co-aggregation of the solute with hydrotrope aggregates is considered to be essential for its solubilization, the hydrotropes with aromatic ring structure are expected to show better solubilization of curcuminoids.

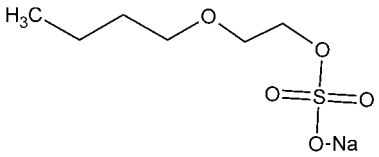
The solubility of total curcuminoids in different hydrotrope solutions at pH 7 was studied to determine efficiency of hydrotropes for solubilization. The solubility data at 30°C are shown in Fig. 3. In all hydrotrope solutions increase in the solubility of curcuminoids was observed to different extents and also beyond a certain concentration of each hydrotrope, which is characteristic of that hydrotrope. For example, both Na-Sal and PTSA solutions showed very low solubility of curcuminoids, even at concentrations as high as 3–4 mol/dm^3. Na-BMGS showed more efficient solubilization of curcuminoids though it does not contain any aromatic ring. On the other hand Na-NBBS solutions showed a substantial increase in the solubility, even at concentrations as low as 0.1 mol/dm^3. At concentrations close to 1.0 mol/dm^3, Na-NBBS solutions could dissolve almost 8 grams of curcuminoids in 1 dm^3 of solution. Curcuminoids are practically water insoluble otherwise. Na-CuS solutions showed a slightly weaker solubilization effect than Na-NBBS but a much larger improvement than those shown by remaining three hydrotropes. For Na-CuS solutions, however, the increase in solubility was more significant beyond 1 mol/dm^3. It must be noted that although these aromatic hydrotropes were selected on the basis of premise that curcuminoids being phenolic would be dissolved well by aromatic hydrotropes, the results indicated that aromatic ring interaction might not be solely responsible for the hydrotropic effect. The only distinguishing feature amongst these aromatic sulfonates is the alkyl chain on the aromatic ring, and Fig. 3 is clearly indicative of the possible

Aromatic sulphonate



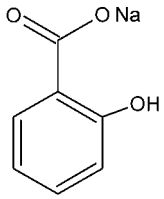
Na-NBBS Na-CuS PTSA

Aliphatic sulphate



Na-BMGS

Aromatic carboxylate



Na-Sal

Figure 2. Structure of hydrotropes.

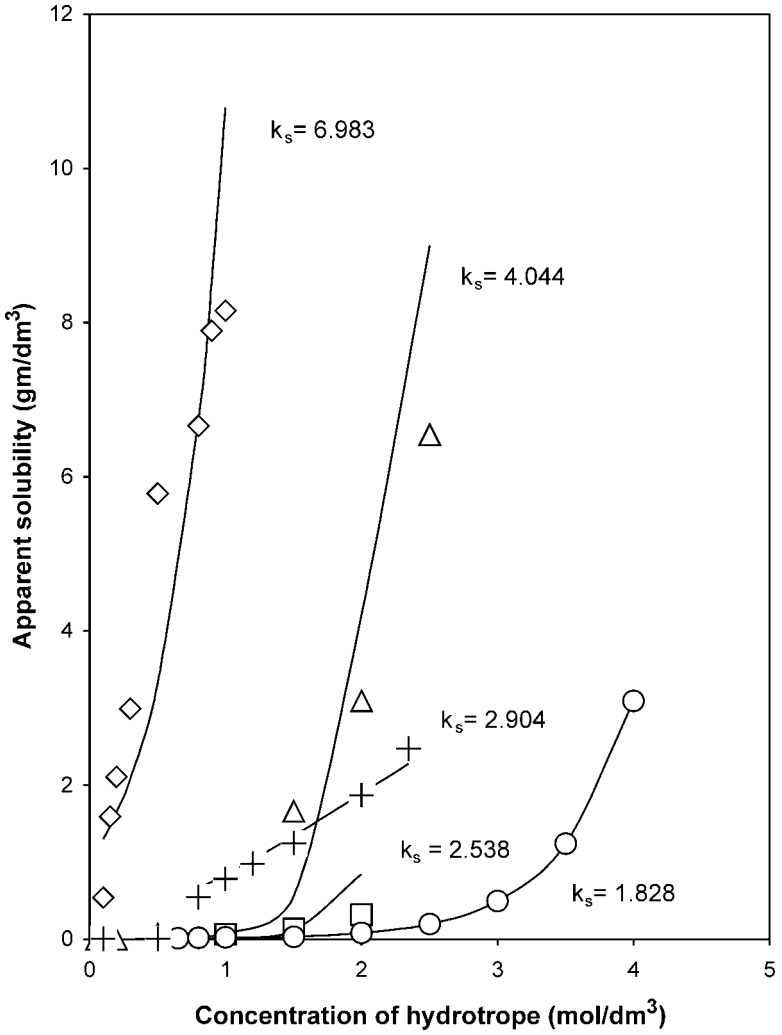


Figure 3. Apparent solubility of curcuminoids in different hydrotropes (temp: 30°C) (data fitted in normal exponential equation): ◇ Na-NBBS; △ Na-CuS; □ PTSA; ○ Na-Sal; + Na-BMGS.

hydrotrope effect for increasing solubilization capacity, from toluene sulfonate to butyl benzene sulfonate. The solubilization also seemed to be very specific as it was observed with Na-NBBS solutions at concentration more than 1.0 mol/dm^3. As concentration of Na-NBBS was increased further, instead of a further increase in the solubility of curcuminoids, precipitation of a solid was observed with a continuous decrease in the solubilized curcuminoids. The precipitated solid was isolated from the solution and was found to contain both curcuminoids and also Na-NBBS on IR spectroscopy. No specific complexation was observed between the two compounds, however. It seems that hydrotrope molecules may form an inclusion-type compound with the solute. The curcuminoids probably promote close aggregation of the hydrotrope molecules, so much so that the aggregates become insoluble in water and precipitate out of the solution. This is probably the first instance where precipitation of a hydrotrope aggregate was observed along with the solute. It must be noted that Na-NBBS otherwise is a highly water-soluble organic salt, while curcuminoids are almost insoluble in water. It may be possible that other hydrotropes may show a similar behaviour with curcuminoids at higher concentrations, and/or similar characteristics of precipitation may be observed with other solutes of appropriate structures.

The solubility of a solute in hydrotrope solutions (s) is usually empirically correlated with the hydrotrope concentration (C_s) using an exponential dependence, in manner analogous to the salt effect on solubility of organic compounds.

$$\ln(s/s_w) = k_s C_s \tag{1}$$

where s_w is solubility of solute in water and k_s is Setchnow constant.

The positive values of Setchnow constant show the salting-in, and attempts were made earlier to predict this value using characteristics of hydrotrope and of solutes.[19,30] However, the prediction for a phenolic solute is off by several factors and cannot be employed in the present case. Figure 3 also shows that although the fit of solubility data in the exponential relation is reasonably good, it is difficult to justify the applicability of the exponential relation to represent solubility data in the hydrotrope solutions. The exponential equation does not take into consideration the aggregation tendency of the hydrotrope molecules and/or co-aggregation of solute molecules with them, and particularly the saturation limits reached at high hydrotrope concentration cannot be predicted.

A recently proposed association model from this laboratory tries to account explicitly for the aggregation behaviour of hydrotrope molecules and subsequent interactions of solute with these hydrotrope assemblies.[30]

The parameters of the model characterize the hydrotrope–hydrotrope and solute–hydrotrope interactions, with assumptions that hydrotrope molecules associate in a stepwise manner to form oligomers and multimers such that the association constant becomes weaker to addition of extra hydrotrope molecules. The association constant for an n-mer is related to dimerization (K_2) constant, i.e., $K_n = K_2/n$.

The concentration of a monomeric hydrotrope molecule (H_1) is related to the total hydrotrope concentration (C_s) through the following equation:

$$C_s = H_1[2\exp(K_2H_1) - 1] \tag{2}$$

On further assumptions that the hydrotrope assemblies can co-solubilize the solute with an n-mer capable to take up a maximum of '(n − 1)' solute molecules and that the solute association with the hydrotrope assemblies becomes weaker on every additional solute molecule in the same manner as the hydrotrope aggregation process, the increase in the solubility can be related to the hydrotrope–solute association constant (K_s) through equation:

$$St = 2(K_S/K_2)[S_1][\exp(K_2H_1) - (1 + K_2H_1) \tag{3}$$

The solubility data were fitted in Eqs. (2) and (3) to estimate two parameters of the solubilization process. The constant K_2 characterizes the hydrotrope aggregation, while K_s characterizes hydrotrope–solute interactions. Though the association model is an approximation of the actual solubilization and does need further refinement, it definitely helps in quantifying the effectiveness of a hydrotrope in solubilizing a particular solute.

Table 1 shows k_s, K_2, and K_s values obtained by fitting the solubility data. The hydrotrope solute association constant K_s was maximum (i.e., 122) for Na-NBBS solutions, indicating the best interaction between Na-NBBS and curcuminoids (Fig. 4). K_s, however, decreased as the chain length attached to the phenyl ring was reduced (i.e., K_s for Na-CuS is 48 and for PTSA is 30), indicating weaker interactions between these hydrotropes and curcuminoids. In the case of Na-Sal it decreased further to 11, indicating still-poorer interaction between Na-Sal and curcuminoids. K_s for Na-BMGS was found to be 44, which shows that interaction between Na-BMGS and curcuminoids is as good as that with Na-CuS. In case of all hydrotropes lower values of the hydrotrope aggregation constant (K_2) showed their poor tendency to aggregate. Considering that these hydrotropes are strongly ionic in nature and should be dissociating completely in aqueous solutions and have weak hydrophobic characters, the magnitude of the association constants should not

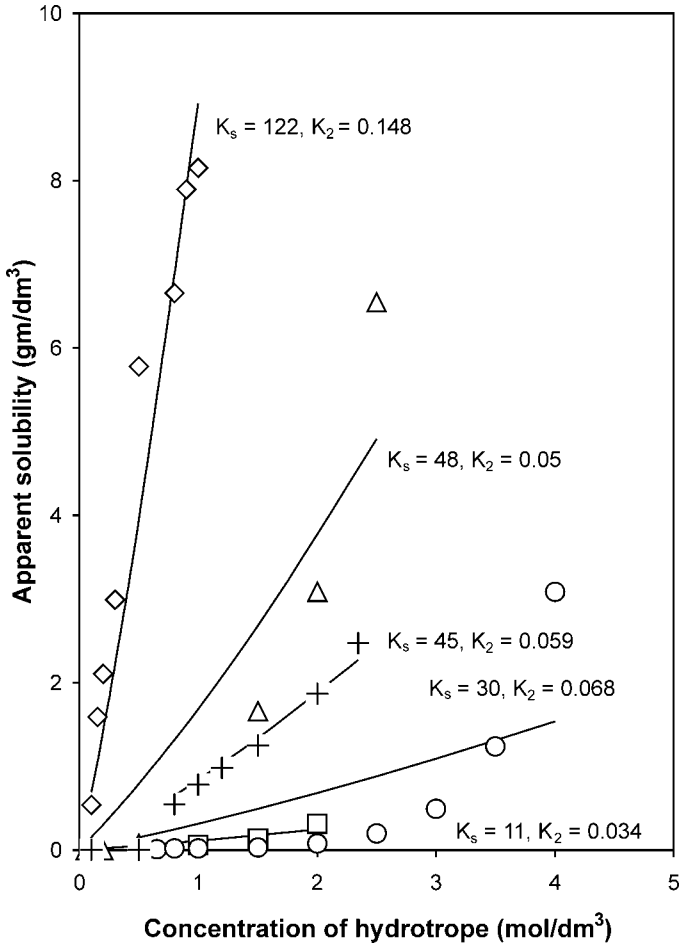


Figure 4. Apparent solubility of curcuminoids in different hydrotropes (temp: 30°C) (data fitted in Association Model): ◇ Na-NBBS; △ Na-CuS; □ PTSA; ○ Na-Sal; + Na-NBBS.

be surprising. It may, however, be possible that association with nonpolar solute may promote a strong aggregation tendency among the hydrotrope molecules.

The parameters of extraction were studied after confirming the usefulness of the hydrotropes on the basis of curcuminoid solubility in hydrotrope solutions.

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Extraction of Curcuminoids Using Aromatic Sulfonates

Three aromatic sulfonates were used as hydrotropes: p-toluene sulfonic acid (PTSA), sodium cumene sulfonate (Na-CuS), and sodium n-butyl benzene sulfonate (Na-NBBS). All three hydrotropes contain $-SO_3^-$ group as the hydrophilic group. In PTSA only one $-CH_3$ group is present in the side chain, and we selected its acid form, which is also a good hydrotrope, for acidic compounds. In Na-CuS, isopropyl group with three carbons is present as the side chain, whereas in Na-NBBS a four-member straight butyl chain is present as the side chain. The efficiency of a hydrotrope in solubilization of organic substances usually increases with its increasing hydrotropic character, and coupled with the solubility data, an increasing extraction was expected for these hydrotropes.

PTSA is a weaker hydrotrope compared to the rest of the hydrotropes. Its interaction with curcuminoids was also weaker than the other two hydrotropes of this group. With 2.0 moles/dm^3 aqueous solution of PTSA only 13% extraction of curcuminoids with 79% purity of recovered curcuminoids was achieved, whereas other two hydrotropes showed a much better efficiency for the extraction of curcuminoids. Na-NBBS was the best hydrotrope among three hydrotropes as it showed the highest affinity toward curcuminoids. Although the best hydrotrope for dissolving curcuminoids, Na-NBBS was not the best hydrotrope for the extraction process because of the reason discussed earlier. Na-CuS proved to be a better hydrotrope for extraction of curcuminoids from *Curcuma longa*. It gave good yields of curcuminoids with very high purity. In general as hydrotrope concentration was increased the solubility of curcuminoids also increased. However, at very high hydrotrope concentrations other disadvantages, such as decrease in apparent purity of product, increased viscosity of the suspension, and formation of thick paste with raw material, became prominent.

Extraction of Curcuminoids Using Na-NBBS Solutions

Extraction by aqueous solutions of Na-NBBS gave deeply orange colored extract solutions for all concentrations. The intensity of the color increased from lower concentration of the hydrotrope to its higher concentrations. As expected from the experience with hydrotropic extraction, this extract phase was diluted with water. The precipitation of curcuminoids from this extract was expected on dilution. However, to our surprise there was no immediate precipitation of curcuminoids on dilution. The increasing dark color of the extract solution was a clear indication of the increased solubilization of

curcuminoids. However, the recovery did not seem to be facilitated by simple dilution by water, and many days were needed for appreciable recovery by precipitation.

The observed anomaly in the behaviour of Na-NBBS can be explained on the basis of very strong interaction between Na-NBBS and curcuminoids. The solubilization of the curcuminoids by Na-NBBS was highly cooperative and influenced by nonpolar interactions between the aromatic ring and hydrocarbon structure of curcuminoids with the hydrotrope. Because of very high affinity at higher concentrations of Na-NBBS, it probably forms inclusion complex with curcuminoids.

This great affinity restricts the recovery of the curcuminoids from Na-NBBS solutions by mere dilution using water and pH adjustment became necessary to break the association between Na-NBBS and curcuminoids for faster recovery by precipitation. Being acidic, curcuminoids can be precipitated by addition of an acidic solution by bringing the solution pH below their pKa. As such the pH of hydrotrope solution was neutral and below the pKa of the phenolic species. Addition of acid solution to adjust pH at 3, however, still did not give any immediate precipitate. We, therefore, first adjusted the pH of the solution to 9 and then within 10 minutes the pH was lowered to 3, as curcuminoid degradation rate is very high above pH 7.5, which gave a yellow colored precipitate. However, the precipitate was not completely soluble in any solvent or water. On further treatment of this precipitate with alcohol it could be fractionated into two parts: one alcohol insoluble part, which seem to be mostly starchy material, and the second alcohol soluble part, which gave the characteristics of curcuminoids with purity of 95%. It also appears that solubilization of curcuminoids by Na-NBBS involves formation of a specific inclusion complex, which depends on the pH of the surrounding medium.

During the extraction of curcuminoids from the *Curcuma longa* particles, the degree of extraction could be very high because of the very high affinity of curcuminoids toward Na-NBBS. However, the extracted curcuminoids may simply precipitate as complex and, therefore, are filtered off along with the raw material while separating the solution by filtration. This undoubtedly decreases the recovery of curcuminoids from the extract.

Figure 5 shows the rate of extraction of curcuminoids into Na-NBBS solutions (1.0 mol/dm^3) under identical conditions at four different temperatures in the range 30°C to 60°C. The rate of extraction in the initial period is similar, giving a slightly higher rate at higher temperatures, but in the later stages the difference in the rates of extraction and the extents of extraction is substantial. The extraction also

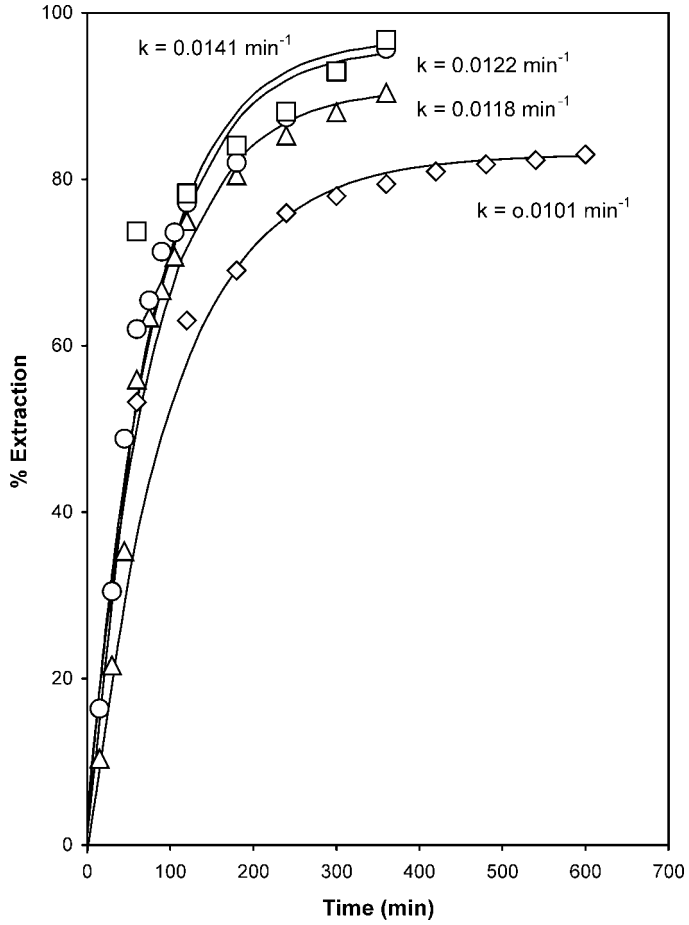

Figure 5. Effect of temperature on extraction kinetics using Na-NBBS (Na-NBBS conc: 1.0 moles/dm^3, solid loading: 5% w/v): ◇ 30°C; △ 40°C; ○ 50°C; □ 60°C.

seems to follow a first-order dependence. The extraction efficiency is defined in term of the percent of curcuminoids extracted from that present in the raw material.

The extraction data obtained were fitted in first-order kinetic equation to estimate extraction rate constant (k), the reciprocal of which represents the characteristic time of extraction, i.e., a higher value of 'k' should correspond

to the higher rate of extraction.

$$\% \text{ extraction} = b\,(1 - e^{-kt}) \tag{4}$$

where

t = time

k = extraction rate constant, time^{-1}

b = maximum extraction achieved at the specified conditions

The data were fitted in Eq. (4), and the calculated rate constants are reported in Fig. 5. The rate constants increased on increasing the temperature of the extraction.

Extraction of Curcuminoids Using Na-CuS Solutions

Extraction of turmeric using aqueous Na-CuS solutions also gave deep yellow colored extract solutions for all concentrations of the hydrotrope. When this extract solution was diluted with water at room temperature of 30°C and the pH was adjusted to 3, it gave yellow colored precipitate. The precipitate was soluble in methanol and contained mainly curcuminoids with the purity of 90%.

The effect of concentration of Na-CuS on the extraction of curcuminoids was then studied to optimize the conditions for extraction. The hydrotrope concentration was varied in the range of 0.5 to 2.0 moles/dm^3. Figure 6 shows a sharp increase in the extraction at 0.5 to 1.0 moles/dm^3 Na-CuS concentration, after which the extraction remained almost constant. The purity of recovered curcuminoids remained above 95% but showed a slight decline at higher hydrotrope concentrations.

The slight decrease in the extraction of curcuminoids after 1.0 moles/dm^3 hydrotrope concentration can be attributed to a similar complex formation between Na-CuS and the curcuminoids as observed in the case of Na-NBBS. But the affinity of Na-CuS toward curcuminoids was not as strong as that with Na-NBBS; also no precipitation was observed in the solubility experiments. However, the purity of recovered curcuminoids remained very high. The Na-CuS solution at 1.0 moles/dm^3 concentration gave 47% recovery of curcuminoids with 96% purity. In order to further study other parameters of extraction, the concentration of Na-CuS was restricted to 1.0 moles/dm^3.

Three particle sizes (6 # mesh, 22 # mesh, and 85 # mesh) of the solid raw material were studied for their effect on the extraction efficiency using

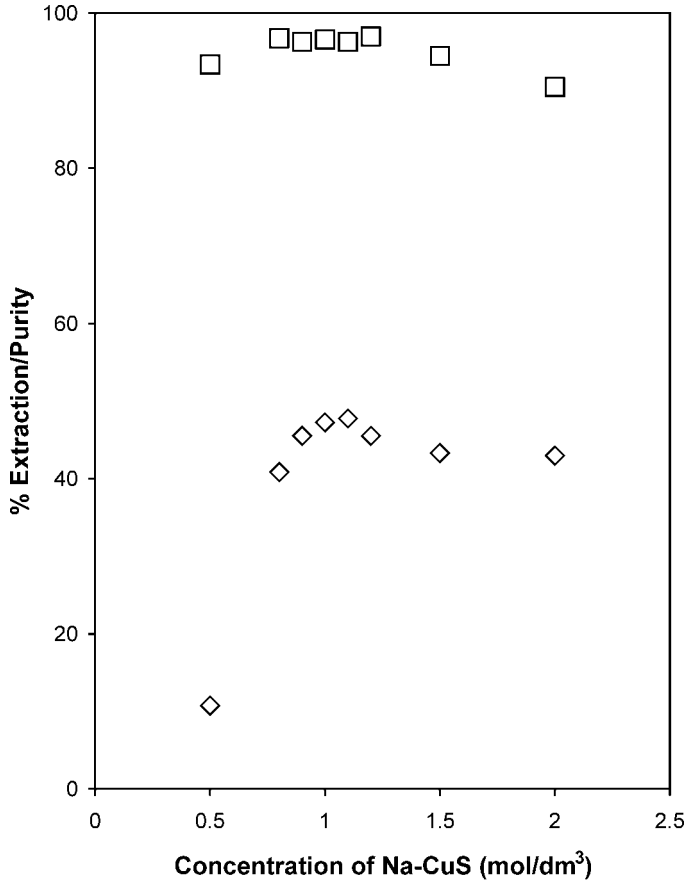

Figure 6. Effect of concentration of Na-CuS on extraction of curcuminoids (temp: 30°C, solid loading 5% w/v): ◇ % extraction; □ % purity.

Na-CuS solutions. With smaller particle size the extraction of curcuminoids increased substantially but at the cost of purity of the recovered curcuminoids precipitated from solution by dilution at pH 3.0. With 6 # mesh particle size, 49% extraction of curcuminoids with 95% purity was observed. With 22 # mesh and 85 # mesh size particles the extraction increased to 55% and 64%, respectively, but the purity decreased to 90% and 87% at the same time. Also other problems, like difficulty in filtering the extract because of formation of thick paste of raw material with extractant solution, were observed with finer particle size. For 85 # mesh particle size of raw material, it was extremely

difficult to use even a 5% w/v loading and the loading had to be decreased to 3% w/v. Coarse raw material is, therefore, preferred to get good yields of curcuminoids with higher purity and to have less operational problems.

Figure 7 shows the percent extraction of curcuminoids with time at temperatures of 30°C, 40°C, and 50°C. The percent extraction increased sharply first but the rate of extraction decreased gradually in first 4 hours. The rate of extraction is similar, giving a slightly higher rate at higher temperatures. The extraction also seems to follow a first-order dependence.

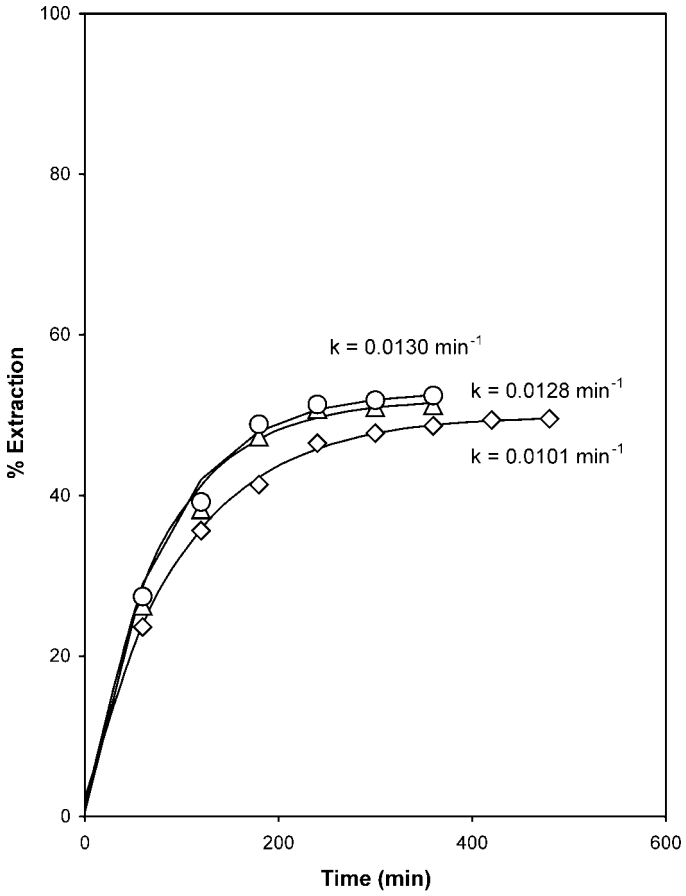

Figure 7. Effect of temperature on extraction kinetics using Na-CuS (Na-CuS conc: 1.0 moles/dm^3, solid loading: 5% w/v): ◇ 30°C; △ 40°C; ○ 50°C.

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The data were fitted in Eq. (4), and rate constants were found to be of magnitude similar to those obtained with Na-NBBS solutions. The lower percent of extraction, about 50%, seems to be the consequence of solubility limit of curcuminoids in the hydrotrope solutions.

Extraction of Curcuminoids Using Na-BMGS Solutions

Sodium salt of butyl mono glycol sulfate is a short chain aliphatic sulfate with butyl chain attached to mono glycol with $-SO_3^- Na^+$ group on other side. The $-SO_3^-$ group is hydrophilic group as in aromatic sulfonates, and the four-member linear butyl chain is hydrophobic in nature. Na-BMGS has no phenyl ring or very long carbon chain, yet it is an excellent hydrotrope with better solubilizing properties than some of the other hydrotropes. The hydrotropic property of Na-BMGS indicates that aromatic ring is not a prerequisite for an organic salt to function as a hydrotrope.

Extraction of *Curcuma longa* using Na-BMGS solutions also gave deep yellow to orange colored extracts. The intensity of the color increased with the increase in concentration of hydrotrope obviously because of the greater amount of curcuminoids dissolved in the solution. When the extract solutions were diluted using water at room temperature, it gave yellow colored precipitate, even without any pH adjustment unlike that in the case of Na-CuS. The precipitate was soluble in methanol and contained mainly curcuminoids.

The effect of concentration of Na-BMGS on the yield of curcuminoids is shown in Fig. 8. The extraction of curcuminoids increased almost exponentially beyond 0.8 moles/dm^3 of Na-BMGS concentration. However, the extraction decreased after its concentration of 1.5 moles/dm^3 in a manner similar to that observed with the aromatic sulfonates. Figure 8 also shows an increase in the purity of recovered curcuminoids up to 1.0 moles/dm^3 concentration and then it remained constant, even at higher concentrations of hydrotrope.

The decrease in extraction after 1.5 moles/dm^3 concentration of Na-BMGS probably shows the effect of increased viscosity of suspension due to the increased hydrotrope concentration. The increased viscosity of suspension decreases the efficiency of mixing, which leads to poor penetration of hydrotrope solution through the raw material. The swelling of the raw material also retains a substantial amount of the solution within the filter cake, thereby reducing the overall efficiency of the extraction.

The effect of particle size of the raw material on efficiency of curcuminoid extraction was studied using raw materials of mesh size #6, mesh

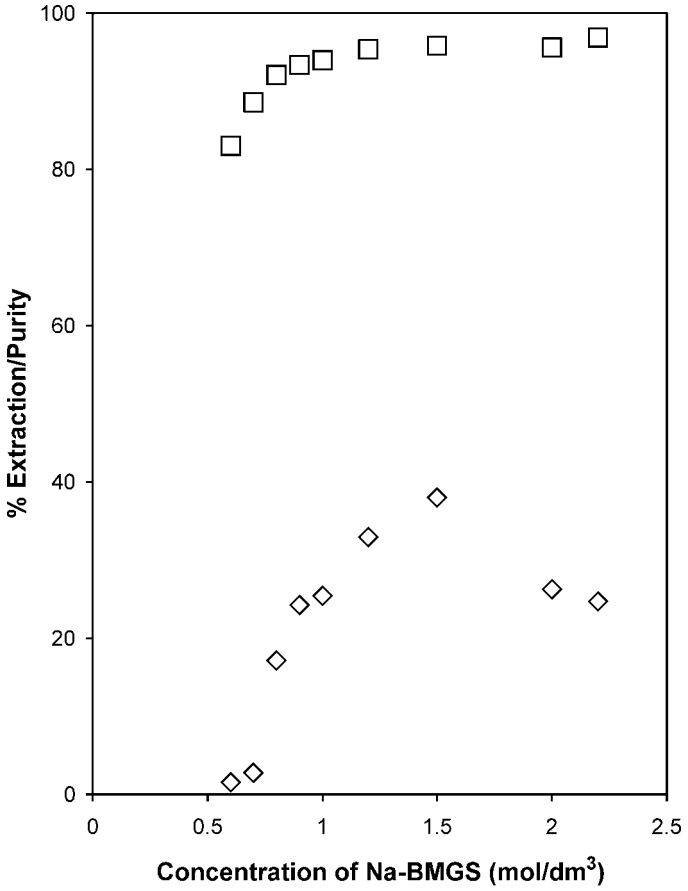


Figure 8. Effect of concentration of Na-BMGS on extraction of curcuminoids (temp: 30°C, solid loading: 5% w/v): ◇ % extraction; □ % purity.

size #22, and mesh size #85. It was observed that extraction of curcuminoids increased marginally from 42% to 48% as mesh size was increased from #6 to #22, but after that the extraction considerably decreased from 48% to 39% as the mesh size was increased from 22 to 85. The decrease in extraction with the increasing mesh size can be due to increased viscosity of suspension due to finer raw material particles, which create mixing problems. In all three cases, however, the purity of recovered curcuminoids remained very high. The mesh size of #22 was selected as optimum size of raw material for further studies

using Na-BMGS as hydrotrope. The degree of percent extraction can be improved by recycling the solid filter cake with fresh hydrotrope solution.

The kinetic studies using Na-BMGS were done at two temperatures, 35°C and 50°C. The rate of extraction in initial period was similar, giving slightly higher value at 50°C, but in the later stages the difference in the rates of extraction was substantial (Fig. 9). The extraction also seemed to follow a first-order dependence. The rate constants increased on increasing

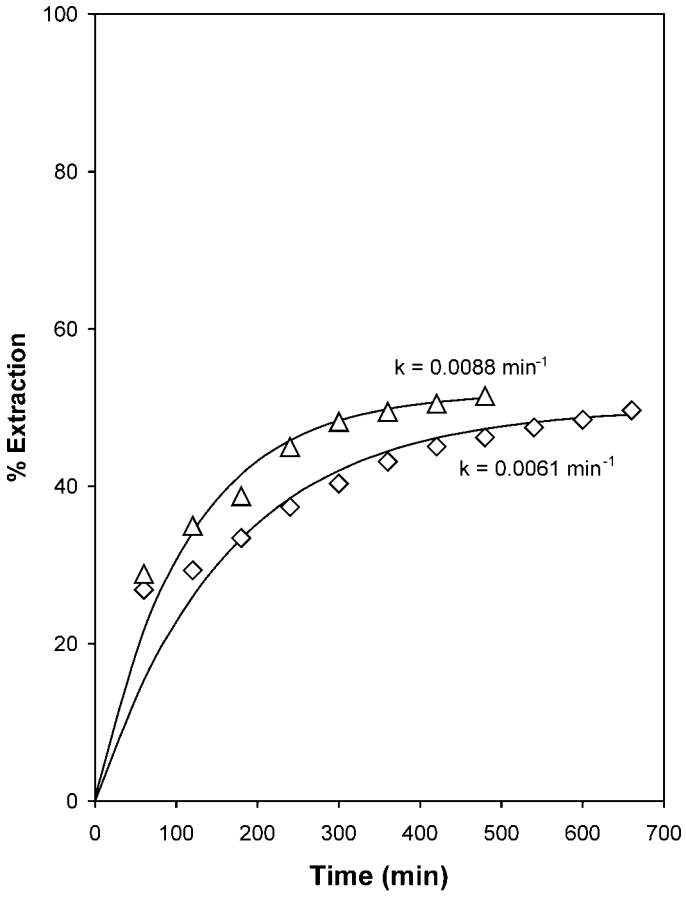


Figure 9. Effect of temperature on extraction kinetics using Na-BMGS (Na-BMGS conc: 1.5 moles/dm^3, solid loading: 5% w/v): ◇ 35°C; △ 50°C.

the temperature; however, the values were substantially lower as compared to the rates obtained with the aromatic sulfonates.

Extraction of Curcuminoids by Sodium Salicylate Solutions

Sodium salicylate is a widely used hydrotrope in pharmaceutical formulations to dissolve insoluble drugs. It consists of no side chains but the —OH group in *ortho* position probably supports the aggregation process. Extraction of *Curcuma longa* by Na-Sal solution gave a yellow colored extract. On dilution with water the extract gave a yellow methanol-soluble precipitate. The precipitate contained mainly curcuminoids.

The effect of Na-Sal concentration on extraction of curcuminoids was studied in the range of 0.4 to 4.0 moles/dm^3. The extraction was very poor up to 1.5 moles/dm^3 and then increased sharply and reached to maximum of 27% at 3.0 moles/dm^3 and remained constant thereafter (Fig. 10). Na-Sal, being a relatively weak hydrotrope, required much higher concentrations, even beyond 1.5 moles/dm^3, to give an appreciable extraction of curcuminoids. The purity of recovered material also remained very high (above 90%) but showed a drastic decrease to 77% at 4.0 moles/dm^3 concentration (Fig. 10).

The effect of particle size of raw material on extraction of curcuminoids was studied using three different particle sizes, as mentioned in the previous hydrotrope studies. Na-Sal extraction gave almost the same results at three particle sizes of *Curcuma longa* as with NaCuS. The purity of the recovered curcuminoids also remained almost the same at 90%. Thus coarse particles were selected for further extraction studies.

Using the optimum concentration of Na-Sal (3.0 moles/dm^3) and 5% w/v loading of the raw material, the rate studies were conducted at temperatures of 30°C and 40°C. Figure 11 shows percent curcuminoids extracted with respect to time at both temperatures. The rate of extraction of curcuminoids increased with time and with temperature. The extraction also seemed to follow the first-order dependence in a manner as the other hydrotropes. The rate constant increased on increasing the temperature. Beyond 40°C the increase in the rate of extraction was not very significant. Also the purity of recovered curcuminoids decreased rapidly beyond 40°C.

Effect of Hydrotropes on *Curcuma Longa* Cell Structure

To understand the mechanism of hydrotropic extraction of curcuminoids from *Curcuma longa* cells, intact rhizomes were studied microscopically

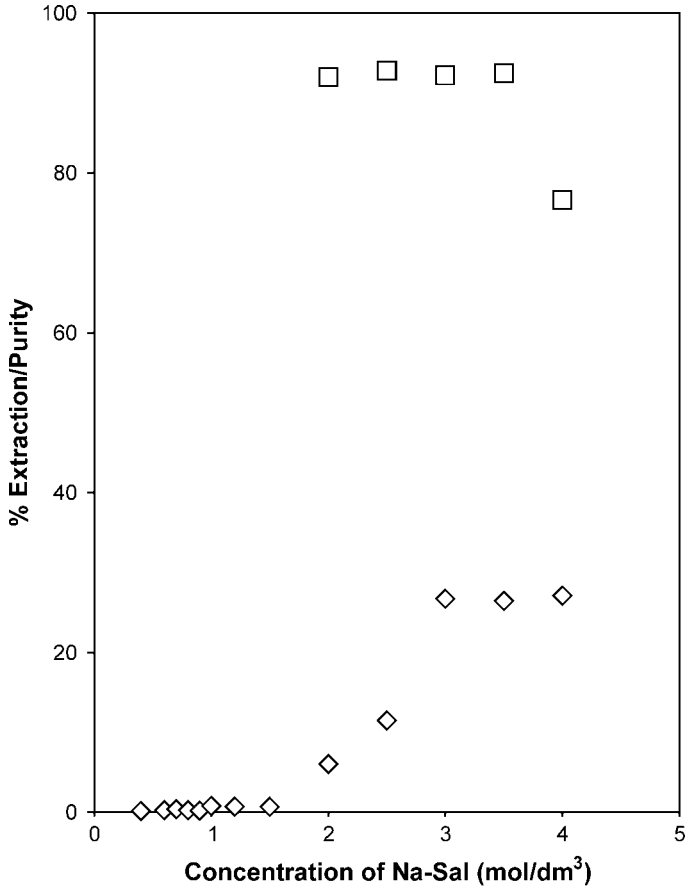


Figure 10. Effect of concentration of Na-Sal on extraction (temp: 30°C, solid loading 5% w/v): ◇ % extraction; □ % purity.

to monitor the hydrotrope action on the cellular structures. The broken/pulverized rhizomes were also exposed directly to aqueous hydrotrope solutions to monitor the hydrotropic effect on oleoresin cells.

In hydrotropic extraction of turmeric, turmeric rhizomes were pulverized to obtain certain mesh size powder. In the process the outer covering of epidermis, hypodermis, and cork cells gets disturbed, and the oleoresin cells containing curcuminoids can be directly exposed to hydrotrope solutions.

The cell wall structure consists of phospholipid bilayers. The ability of hydrotrope to disturb the lamellar crystal structure of surfactant also helps in

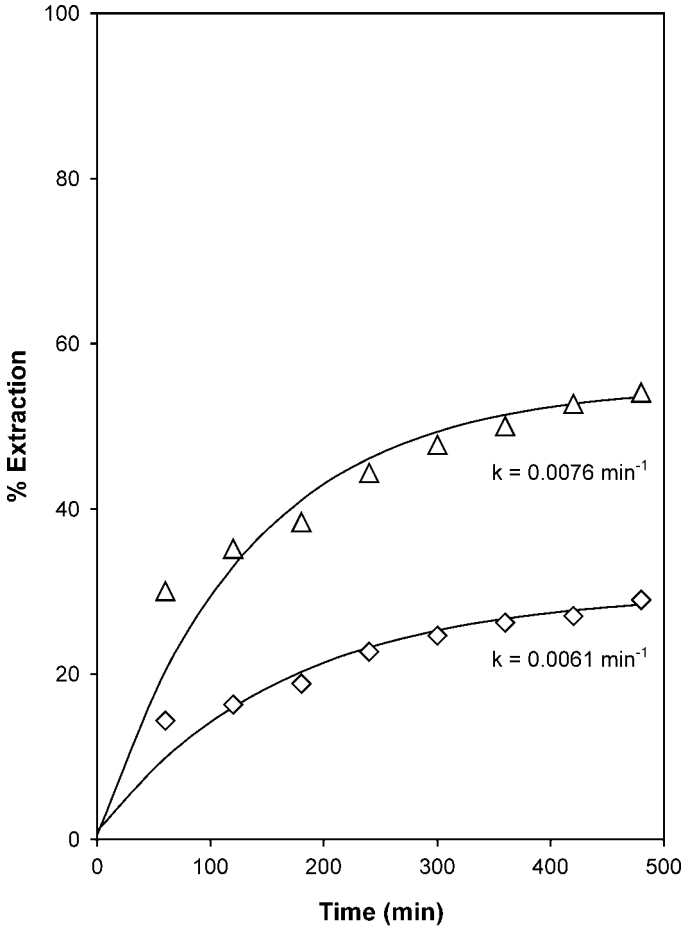
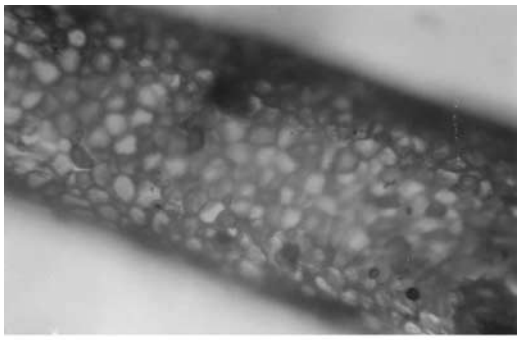


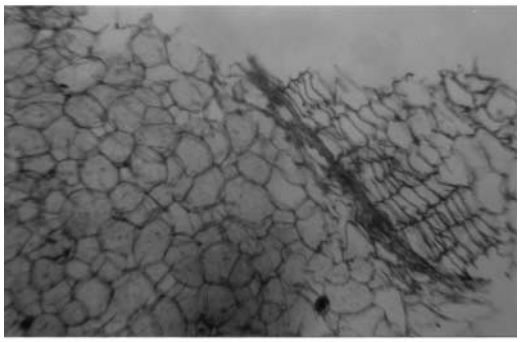
Figure 11. Effect of temperature on extraction kinetics using Na-Sal (Na-Sal conc: 3.0 moles/dm^3, solid loading: 5% w/v): ◇ 30°C; △ 50°C.

disturbing phospholipid bilayer, and the solution penetrates the cell wall. The extraction process, therefore, utilizes the ability of hydrotrope molecules to penetrate the cell wall and to make curcuminoids available for faster dissolution in the aqueous solutions. The selectivity of hydrotrope toward the curcuminoids in the cell is probably because of the phenolic nature of curcuminoids.

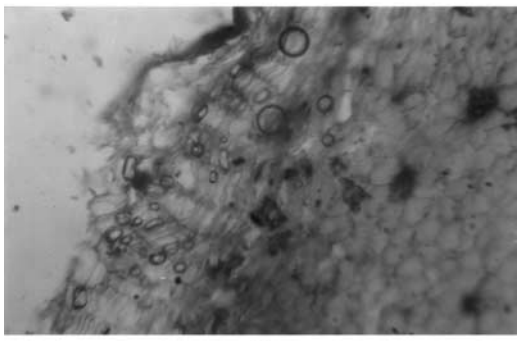
Typical microscopic observations of *Curcuma longa* cells are showed in Fig. 12a–c. The intake of water by the turmeric cells swells the rhizome.



(a)



(b)



(c)

Figure 12. (a) Turmeric rhizome cells. (b) Effect of aqueous Na-CuS solution on turmeric rhizome cells. (c) Effect of aqueous Na-BMGS solution on turmeric rhizome cells.

The water soaking showed very small effect on the cork cells. But the hydrotrope solutions first disturbed the organized median suberin lamella and then the mature cork cell. The cork cell layers could be seen as disturbed when exposed to hydrotrope solutions. The cork cell layers are twisted and shattered, and the hydrotrope solution can therefore penetrate into the inner structure. The degree of disturbance depends on nature of the hydrotrope. Na-CuS almost completely shattered the cork cell layers. Na-BMGS broke them to a less extent but more twists were observed, whereas in case of Na-Sal or PTSA a less prominent effect was observed.

When the inner part of rhizomes gets exposed to hydrotrope solutions, it not only swells the cells but also creates gaps in the cellar structure. The solution attacks the cells from all sides and penetrates into it. The effective penetration depends on the nature of hydrotrope used. Na-CuS and Na-BMGS were observed to be more effective, whereas Na-Sal and PTSA were poor in their action. These microscopic observations explain the difference in extraction efficiencies of the hydrotropes. Na-CuS and Na-BMGS gave the highest extraction of curcuminoids with the highest purity, followed by PTSA and Na-Sal.

CONCLUSION

Extraction of curcuminoids using hydrotropy showed various advantages over conventional solvent extraction processes and also on supercritical fluid extraction. This method is simple, fast, and easy to set up. The yield of curcuminoids obtained in this process is comparable with conventional process, whereas the purity of the product is far higher than curcuminoids obtained by conventional solvent extraction processes. In this process no chemical action takes place between hydrotrope and curcuminoids, thus diluted hydrotrope solution may be recycled after concentrating it by evaporation. If necessary a charcoal treatment may be given to remove other pigments from the raw materials before recycling the hydrotrope solution. No contamination of the product is possible as no organic solvents are involved. The presence of a large amount of water also eliminates fire hazards and other related accidents. Traces of hydrotrope from curcuminoids can be removed by simple water wash, as hydrotropes are highly water-soluble.

Thus extraction of curcuminoids from *Curcuma longa* rhizomes using hydrotropy is an environmentally and industrially safe and economical process.

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