Extraction of Piperine from *Piper nigrum* (Black Pepper) by Hydrotropic Solubilization

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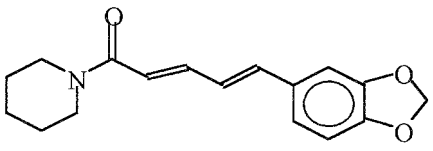
Hydrotropes, such as sodium alkyl benzene sulfonates and sodium butyl monoglycol sulfate, were used for the selective extraction of piperine by cell permeabilization of *Piper nigrum* fruits. Penetration of the hydrotrope molecules into the cellular structures and subsequent cell permeabilization were hypothesized to explain the enhanced extraction rates of aqueous hydrotrope solutions. Hydrotrope molecules, after adsorption on a cell wall, cause disorder in its structure and in the bilayered cell membrane to facilitate the rapid extraction of piperine. The hydrotrope solution showed selective and rapid extraction of piperine from black pepper. The recovered piperine was ∼90% pure and substantially free from oleoresins. The type and nature of the hydrotrope, the hydrotrope concentration, the temperature, and the particle size all had significant effects on the extraction process.

Introduction

The increased interest in plant-derived drugs in recent years is because of their undisputed efficacy as phytomedicines and because active principles from natural products serve either as templates or as intermediates for synthetic drugs.[1] Despite the sophistication of modern organic synthesis, it is not always economically feasible to synthesize drugs that are similar to these active ingredients. Accordingly, most plant drugs are cultivated and are used clinically as semipurified or purified extracts. The extraction and purification steps can constitute 50−90% of the final product cost in such cases.

Piperine (structure 1), which is a major alkaloid in black pepper,[2] exhibits a potent chemo-protective effect against procarcinogens and also bacteriostatic, fungistatic, and insecticidal activities.[3] Piperine provides protection against seizures in epilepsy and has been gaining increasing attention as a bioavailability enhancer in the formulations of several drugs.[4,5] Piperine, because of its protective effect against radiation, can also be administered to cancer patients before radiotherapy.[6] These applications suggest a need for pure piperine that is free from residual solvents to enable its direct use in medicinal formulations.



PIPERINE

On an industrial scale, pepper is comminuted into flakes or ground into coarse powder and then extracted repeatedly with an organic solvent such as acetone, ethanol, or chlorinated hydrocarbons.[7] Repeated solvent extraction of raw pepper particles for long durations results in the extraction of other components, such as polysaccharides, gums, and non-flavor substances. As a result, the solvent extraction processes usually give complex crude products. The crude extract has to then be purified by multistep techniques such as chromatography or crystallization. Apart from the poor extract quality, difficulties in handling large volumes of inflammable volatile organic solvents and residual solvent traces remaining in the final product limit the use of organic solvents for pepper extraction.

Supercritical fluid extraction using carbon dioxide is another option for the extraction of piperine.[8,9] The cost of the high-pressure equipment needed to obtain supercritical extraction conditions, however, becomes prohibitively high and limits the application of supercritical extraction to only high-value and low-volume materials. High-pressure steam treatment can also enhance extraction rates by an osmotic shock; however, this technique is relatively slow and consumes a large amount of steam.[10] Ultrasound treatment has been claimed to increase the yield and mass-transfer rate in several solid−liquid extraction processes.[11] The treatment ruptures the cell walls through strong dynamic stressing, which results from the spontaneous formation of bubbles in a liquid below its boiling point and the collapse of these bubbles within a very short time. The effect of ultrasound is, however, localized, and its application to a large volume of raw material might be energetically inefficient.

In this report, we present a highly efficient process based on the phenomenon of hydrotropy for the selective extraction of water-insoluble phytochemicals from complex natural products. Hydrotropy refers to the ability of highly water-soluble but mildly surface-active amphiphilic organic salts called hydrotropes[12] (structure 2) to increase the solubility of sparingly soluble or water-insoluble organic compounds in aqueous solutions.

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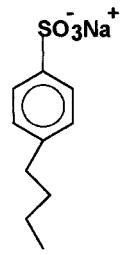
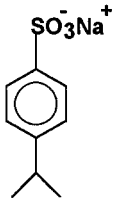
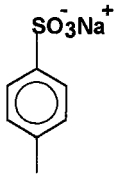
10.1021/ie0107845 CCC: $22.00
Published on Web 05/18/2002



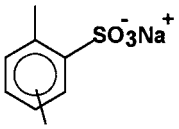
NaNBBS



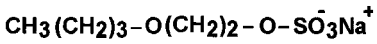
NaCS



NaPTS



NaXS



NaBMGS

Hydrotropy is a collective molecular phenomenon similar to micellar solubilization but with a much higher capacity.[13] It is a consequence of the tendency of amphiphilic hydrotrope molecules to aggregate among themselves and probably with other hydrophobic molecules.[14] These aggregates are supposedly much smaller than surfactant micelles and far less cooperative. Another distinguishing feature of hydrotropes, unlike surfactants, is their ability to differentiate among different organic constituents of a mixture, even closely related substances.[12] It is this ability of molecular recognition that should be useful for the preferential extraction of a compound from naturally occurring raw materials. The high solubilization capacity of hydrotrope solutions should lead to high extraction capacities for otherwise insoluble organic-active elements.

Hydrotropes demonstrate a remarkable property of disrupting the lamellar crystalline structure of surfactants in aqueous solutions, producing a continuous isotropic liquid solubility region.[13] In surfactant solutions, the presence of such a lamellar structure at high concentrations limits the solubility of hydrophobic compounds. These structures are analogous to the phospholipid bilayers of cell membranes. We demonstrate here the ability of hydrotropic solutions, in an analogous manner, to disrupt plant cell structures and aid in the extraction of hydrophobic constituents from the complex biomatrix. The hydrotropic effect is significant above a minimum hydrotrope concentration (MHC) that is a characteristic of a given hydrotrope, analogous to the critical micelle concentration (CMC) of a surfactant. However, because hydrotropes have relatively short hydrocarbon chains or hydrophobic groups, their MHCs are usually in the molar range. The solubility of an organic compound in a hydrotrope solution rises almost exponentially immediately above the MHC, but at higher concentrations of the hydrotrope, it might level off to a plateau depending on the nature of the solute. Dilution of the saturated solution with water is usually sufficient to recover the dissolved solute, which separates out as another solid or liquid phase when the hydrotrope concentration drops below its MHC.

In this paper, we explore the extraction of piperine from *Piper nigrum* using hydrotropic solubilization. The objective of the present work was to develop an efficient process for the extraction of active ingredients from natural products and to understand the role that each parameter plays in the extraction. Disruption of the cellulosic cell wall of the biomatrix and subsequent disorganization of the phospholipid bilayers by the hydrotrope molecules, followed by dissolution of the cellular contents, seem to be the key steps in this extraction process. A high extraction efficiency, large capacity of hydrotrope solutions, and high selectivity toward piperine are major observations.

We believe that hydrotropic extraction can provide a competitive alternative to supercritical fluid extraction. The scale of operation and the ease of scale-up of hydrotropic extraction are at levels unimaginable for supercritical fluid extraction. What is achieved with pressure in the supercritical fluid extraction can be achieved with ease using the hydrotrope concentration in aqueous solutions. Because the solubility enhancement is insignificant at lower hydrotrope concentrations, simple dilution by water provides an easy recovery method, just as does the release of pressure in supercritical fluid extraction. Because hydrotropes are highly water-soluble salts, contamination of the product by hydrotrope molecules is minimal and can be reduced to below an acceptable level, if any, simply by washing with water. We also believe that this method should work with similar efficiencies for other natural products also. In addition, it would be interesting to understand how hydrotropes differ from surfactants in their action on cellular structures.

Experimental Section

Materials and Experimental Methods. The aromatic sulfonate hydrotropes sodium xylene sulfonate (NaXS; mixed isomers with an ethyl benzene sulfonate content of about 6%, as quoted by the manufacturer), sodium cumene sulfonate (NaCS), and sodium *p*-toluene sulfonate (NaPTS), were purchased from Navdeep Chemicals Ltd., Mumbai, India, and were recrystallized with methanol before the extraction studies. *n*-Butyl benzene was obtained from Herdillia Chemicals Ltd., Mumbai, India. It was sulfonated with concentrated sulfuric acid (98%) and then neutralized with sodium hydroxide to give sodium *n*-butyl benzene sulfonate.[14] Sodium butyl monoglycol sulfate (NaBMGS) was obtained as a 50% (w/v) solution (from Huls, Düsseldorf, Germany). Whole pepper berries were obtained from M/s. Cancor India Ltd., Cochin, India. Dichloromethane (DCM) and methanol (HPLC-grade) were used as solvents for high-performance liquid chromatography (HPLC) analysis. Cetyl trimethylammonium bromide (CTAB) and sodium lauryl sulfate (SLS), obtained from Spectrochem, Mumbai, India, had a manufacturer's stated purity of 99% and were used as received.

Continuous Soxhlet extraction with petroleum ether was initially carried out for 48 h to determine the piperine content of the raw material, which was 4.0% (w/w).

Whole pepper fruits were first pulverized to a coarse powder and then separated into batches of different sizes using mechanical sieves. Particles with an average size 50 μm were used for the extraction studies unless stated otherwise. The extraction experiments were carried out in a fully baffled borosilicate cylindrical glass vessel (9-cm height, 7.0-cm i.d.) equipped with a six-bladed turbine impeller (i.d. 2 cm). This entire assembly was kept in a constant-temperature bath during experimentation. A 10-g sample of ground pepper was added to 0.1 dm^3 of hydrotrope solution of a known concentra-

tion in the range 0.05−3.4 mol/dm^3 in the glass vessel. The suspension was agitated vigorously at 1100 rpm for a period of 2 h at 30° C. The solution was then allowed to settle for another hour and was subsequently filtered under vacuum within 10 min. A clear brown-colored liquid was obtained as the filtrate. The solid residue, which consisted mostly of the starch content of pepper, was soft and pulpy but did not hinder the filtration process. The cake was further washed with hydrotrope solution (0.01 dm^3) of the same concentration as in the extraction stage to remove extract residue adhering to it, if any. The wash solution was added to the final extract.

The filtrate was then diluted with water at 30 °C to bring the hydrotrope concentration below its MHC. Piperine precipitated from the solution as fine crystals over a period of 1 h. The suspension was then centrifuged at 2822*g* for 15 min to separate the solid product from the remaining solution. The precipitate was dried and analyzed for purity using HPLC with a 5-μm Novopak C-18 column. The column was initially rinsed with methanol and DCM and then equilibrated with the eluting solvent (DCM/MeOH 100:4).[15] The column was mounted on a Tosho HPLC chromatograph equipped with a 20-μL loop injector. The mobile phase flow rate was 0.6 mL/min, and the detection wavelength was 343 nm. The analysis was isocratic and was carried out for 15 min.

Extraction of piperine from pepper was also conducted separately with aqueous solutions of CTAB and SLS (0.5 mol/dm^3 concentration) in an identical manner for verification of the extraction ability measurements and for comparison.

Solubilization experiments were separately conducted by suspending pure piperine in aqueous hydrotrope solutions at different concentrations ranging from 0.2 to 2.0 mol/dm^3. The solution was equilibrated with excess solid piperine for several hours at a constant temperature of 303 K using a magnetic stirrer. The suspension was then centrifuged, and the centrifugate was extracted into DCM for analysis. The concentration of dissolved piperine in the solution was estimated spectrophotometrically at 343 nm using a Hitachi UV−visible spectrophotometer.

In a separate experiment, an aqueous hydrotrope extract was carefully weighed and dried. The total inorganic phosphorus content was then estimated by a modified Fiske−Subbarao method[16] using sodium molybdate and hydrazine sulfate at 650 nm. A sample of feed hydrotrope solution of the same concentration was taken and subjected to the same procedure for reference.

To determine whether reducing sugars were present, the hydrotrope extract was treated with Fehlings solution.[17] The amino acid content of the hydrotrope extract solution was estimated qualitatively using Ninhydrin reagent.[18] The hydrotrope extract (2 cm^3) was extracted with butanol, and this butanol extract was used for thin-layer chromatography (TLC). The TLC plate was developed in a butanol/acetic acid/water (4:1:1) mixture. The TLC plate was sprayed with Ninhydrin reagent to determine the presence of amino acids.

Results and Discussion

Figure 1 shows representative chromatograms of standard pure piperine, piperine extracted hydrotropically, and piperine extracted with petroleum ether. Hydrotropically extracted piperine is relatively more pure than petroleum ether extracted piperine where other oleoresins are present in a large percentage. The selective extraction of piperine into aqueous hydrotrope solutions prompted further studies of extraction using different hydrotropes and different operating parameters. The extraction of piperine should be the result of changes in the structure of the pepper cells that occur as a result of the presence of hydrotrope in the solution. Both surfactants and organic solvents are known to cause cell permeabilization, although the exact site and mechanism of their action are a point of debate in many cases.[19] Organic solvents, such as toluene, cause considerable damage to the cytoplasmic membrane, while the outer membrane remains relatively intact.[19] Solvents such as DMSO promote the loss of phospholipids in the cell membrane, whereas cationic surfactants, such as CTAB, act on the phospholipid layer through their hydrophobic chains and are known to disrupt it completely.[19−20]

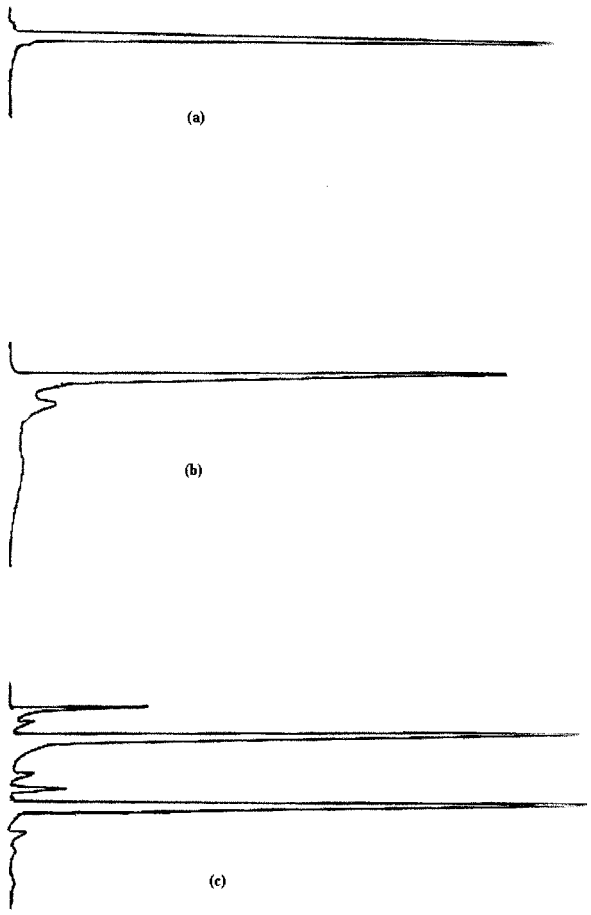


Figure 1. Comparitive HPLC chromatograms of extracted piperine (mobile phase = DCM/MeOH (100:4), flow rate = 0.6 mL/min, detection wavelength = 343 nm): (a) pure piperine, (b) hydrotropically extracted piperine, (c) Soxhlet-extracted piperine

To dissolve piperine, a hydrotrope solution has to penetrate and destabilize the cell structure. Sections of whole *Piper nigrum* fruits were microscopically observed before and after treatment with hydrotrope solutions for 30 min. The images were analyzed with a ProPlus Image Analyzer.

In the case of samples soaked with NaBMGS solution, the cells were very turgid and swollen, but their shape was considerably intact (panels a and b of Figure 2). This suggests an increased permeability of the cell wall, as piperine was extracted from these cells without their

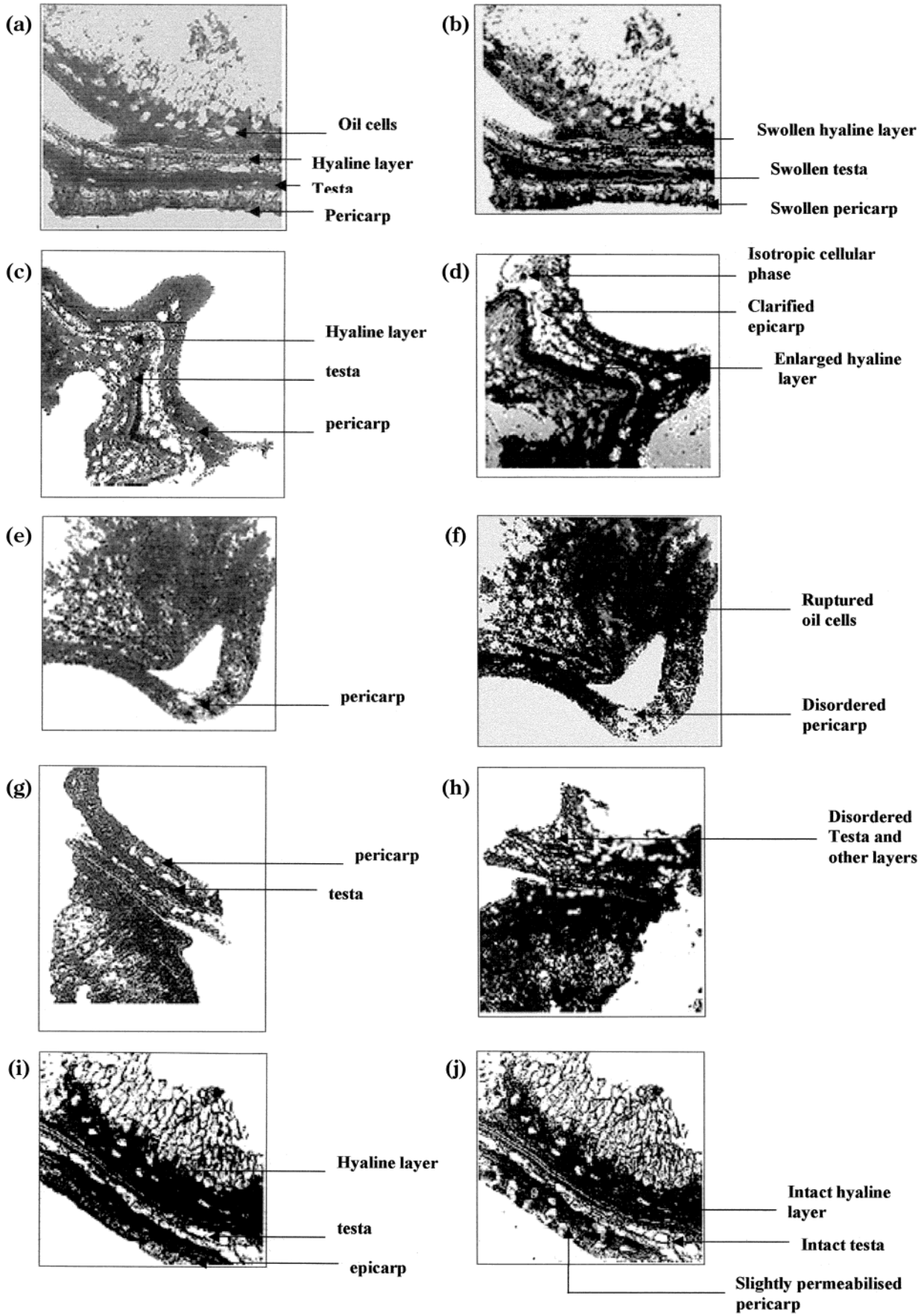


Figure 2. (a) Intact section of *P. nigrum*. (b) Section of *P. nigrum* after soaking in BMGS solution. (c) Intact section of *P. nigrum*. (d) Section of *P. nigrum* after soaking in NaNBBS solution. (e) Intact section of *P. nigrum*. (f) Section of *P. nigrum* after soaking in NaCS solution. (g) Intact section of *P. nigrum*. (h) Section of *P. nigrum* after soaking in NaPTSA solution. (i) Intact section of *P. nigrum*. (j) Section of *P. nigrum* after soaking in NaXS solution.

complete rupture. This should account for the very selective extraction but low yields of piperine obtained with NaBMGS solutions, as described below. In the sections soaked in NaNBBS solutions (panels c and d of Figure 2), three layers of the pericarp appeared clarified. The outer pericarp had a fibrous appearance, indicating slow but definite degradation of the layer. At the edges, the testa, containing a reddish brown pigment and the cellulose layer, seemed to have formed an isotropic phase, with the layers intermixing with each other. Even the hyaline layer of thin walled cells was enlarged. Because the pericarp cells are packed with piperine crystals, a high extraction efficiency and selective extraction were obtained when NaNBBS was used as the hydrotrope, as described below. In the case of NaCS (panels e and f of Figure 2), substantial disruption of the cellular structure and disordering of the pericarp occurred. Cellular debris was visible, along with highly swollen cells. Because the inner part of the pericarp also seemed to have been ruptured, other oleoresins were also extracted from the disrupted oil cells. NaPTS-soaked sections showed an increased rupture of the parenchymatous cells of the mesocarp and the brown pigmented cells of the testa but not of the cells of the pericarp (panels g and h of Figure 2). NaPTS, however, exhibited a low extraction efficiency as brown pigment and other cellular constituents were extracted more than piperine. NaXS-soaked sections (panels i and j of Figure 2) showed only minimal changes in the epidermal layer of the pericarp; even the thick-walled sclereids seemed intact.

From the microscopic observations and preliminary extraction experiments, where a good amount of piperine was extracted, the extraction efficiency of the hydrotrope solutions was clearly evident. For the extraction of piperine, hydrotrope should first adsorb onto the surface of the cells and then penetrate the cell structure to access piperine. The penetrability of the hydrotrope can be related to the parameter[21] pC_{20}/A_{min}, where pC_{20} is the negative logarithm of C_{20}, i.e., the molar concentration of an amphiphile in the aqueous phase required to reduce the surface tension of aqueous solutions by 20 dyn/cm. It is a measure of the surface activity and adsorption efficiency of the amphiphile. A_{min} is the hydrated cross-sectional area under given conditions, which can be estimated from the surface tension data. Although all hydrotropes used in this work have the same polar group, the hydrated cross-sectional area can depend on the manner in which the molecules are packed at the interface or in the aggregate, so the factor pC_{20}/A_{min} can be used to explain penetrability. The pC_{20}/A_{min} values for NaNBBS and NaCS are high at 0.018 and 0.02, respectively, indicating better packing of these molecules in the adsorbed state. For NaXS, NaPTSA, and NaBMGS on the other hand, the pC_{20} values are nearly 10 times lower at 0.0039, 0.0053, and 0.0065, respectively. These values aptly represent the microscopically observed effects of the respective hydrotropes on cellular structures.

The sections taken from the whole fruits soaked in hydrotrope solutions showed broken pericarps and other epidermal layers and the presence of cellular debris. The effects of different hydrotropes on the structure of the plant cells were also different. Because the actual extraction of piperine was carried out using pulverized pepper fruits, there was an initial breakdown of the outer layers of the fruits, leaving the inner epidermal layers directly exposed to the hydrotrope solutions.

Piperine, although present throughout the *Piper nigrum* fruit, is largely concentrated within the pericarp cells. The cell walls, made up of cellulose, present an ordered structure. The primary cell wall consists of glucose polymers, each of which contains roughly 6000 glucose units, whereas in the secondary wall, the number increases to 13 000−16 000 units.[22] Cellulose chains form crystalline structures called microfibrils, each with a diameter of 20−30 nm containing about 2000 molecules in alternate crystalline and noncrystalline sections.[22] The crystalline section forms the three-dimensional cellulose matrix through the formation of the highest possible number of hydrogen bonds. The cell wall as a whole is heterogeneous because it shows two phases on swelling with water and determines the rate of entry and exit of compounds with respect to the cell.[22]

We expect that, as a first step, hydrotrope molecules adsorb on the cellulosic cell wall. The reduction of surface forces at the interface improves the cell wall's wettability, and subsequently, water and hydrotrope molecules can penetrate easily into the cellulosic structure and access the cell membrane.

The cell membrane, responsible, along with the cell wall, for maintaining a balance between the outside bulk solution and the inside of the cell, is made up of phospholipid bilayers interspersed with proteins such as extensins and aquaporins. Extensins are made up of short linear chains of one to four sugar units attached to a polypeptide backbone. The presence of aquaporins in cellular membranes facilitates a trans-cellular pathway for water flow.[23] Two highly hydrophobic hydrocarbons tails of phospholipids create a hydrophobic environment within the membrane. The hydrotrope extract solution showed the presence of inorganic phosphorus (0.0156 mol/dm^3), which indicates probable destabilization of the phospholipid layer.[16] The treatment of the extract with Fehling solution gave a rust-brown-colored precipitate, qualitatively indicating the presence of carbohydrates with reducing groups that reduced cupric ions to cuprous ions.[17] The presence of reducing sugars and amino acids in the extract indicated a partial, if not complete, breakdown of the cell wall polymers and the proteins such as extensins into their respective amino acids.[18] Dissolution of cellulose into the hydrotrope solutions also cannot be ruled out, although it cannot be the sole mechanism of the observed enhanced extraction rates, as it would have caused extraction of other species too into the hydrotrope solutions.

The penetration of hydrotrope into the cell wall and membrane structure probably induces molecular disorganization and alters the permeability of the membrane by dissolving at least some of the cell wall components. We believe that the hydrotrope is capable of this disorganization, as is evident from the disordering of liquid lamellar structures of surfactants in aqueous solutions in the presence of hydrotropes.[24] The liquid lamellar structures are reminiscent of the cellular membrane structures. By inducing a change in the molecular organization of the cell membrane, a hydrotrope alters the permeability of the membrane in such a manner that piperine is made easily accessible to the hydrotrope solution. The schematic representation of the phenomenon of hydrotropic extraction is shown in Figure 3.

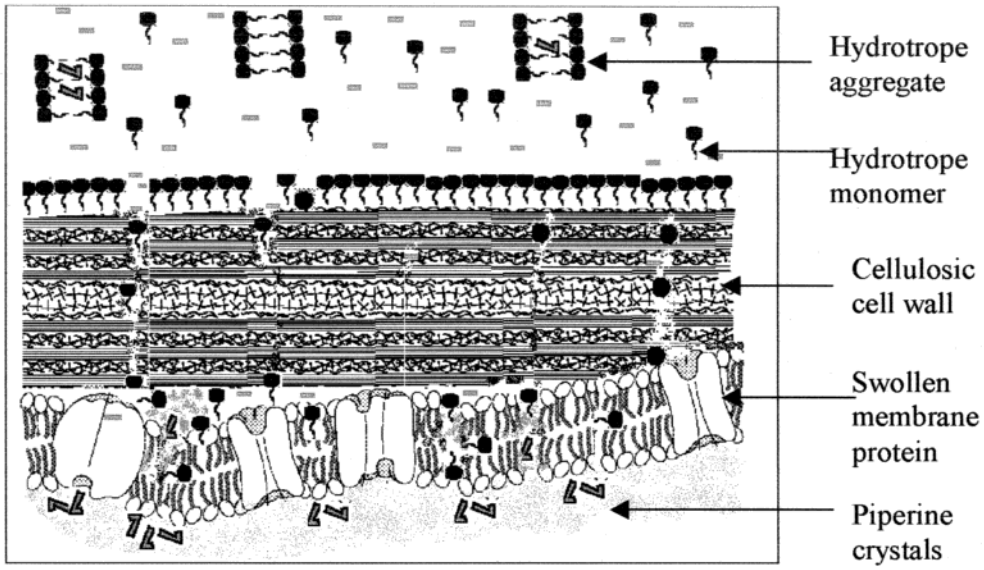


Figure 3. Schematic representation hydrotropic extraction of piperine. On treatment with hydrotrope solution, the water molecules penetrate into the cell wall and through the transport aqua-porins, causing swelling of the membrane proteins, and the hydrotrope monomers also penetrate into the cellular structure. Cell wall and cell membrane disorganization occurs, leading to the release of piperine from within the cell into the hydrotrope solution.

Because hydrotropes show increased solubilities for hydrophobic substances above their respective MHCs, piperine can be easily solubilized and carried into the external hydrotrope solutions. Because of the structural changes in the biomatrix, the rate of extraction of piperine should undergo a substantial enhancement, which indeed is observed. The effects of hydrotropes and their concentrations were investigated further for two different types of hydrotropes, aromatic sulfonates and glycol sulfates.

Substituted aromatic sulfonates such NaNBBS, NaCS, NaXS, NaPTS, and a linear aliphatic sulfate such as NaBMGS have a typical amphiphilic structure, including a strongly ionic hydrophilic group and a hydrophobic group consisting of an alkyl group with or without an aromatic ring. Although the presence of an aromatic ring was once considered essential for the hydrotropic effect,[24] alkyl glycol sulfates defy these expectations and are extremely good hydrotropes. The subtle differences in hydrotropy displayed by the members within each class and between the classes can be attributed to the different sizes of their hydrophobic parts, numbers of $-CH_2-$ groups in the hydrocarbon side chains, and efficiencies of intermolecular packing in their self-aggregates.

Figure 4 shows a distinct relationship between the hydrophobic chain length of a hydrotrope and the efficiency of extraction in the order NaNBBS > NaCS > NaXS > NaPTS. The percentage extraction is defined here as the percentage of piperine initially present in the raw material that was extracted into the hydrotrope solution. The effective hydrophobic chain length varies from C_5 for NaPTS to C_8 for NaNBBS. Hydrotropic solubilization is a collective molecular phenomenon, possibly occurring by the intercalation or co-aggregation of solute with hydrotrope molecules and the self-aggregation of hydrotrope molecules in aqueous solutions, that is a prerequisite for increased solubilization.[13] It is, therefore, not surprising that the solubilization capacity is governed by the hydrophobic functionality, i.e., the alkyl group on the aromatic sulfonates. The hydrophobic volume (v, Å^3) provided by a hydrotrope can

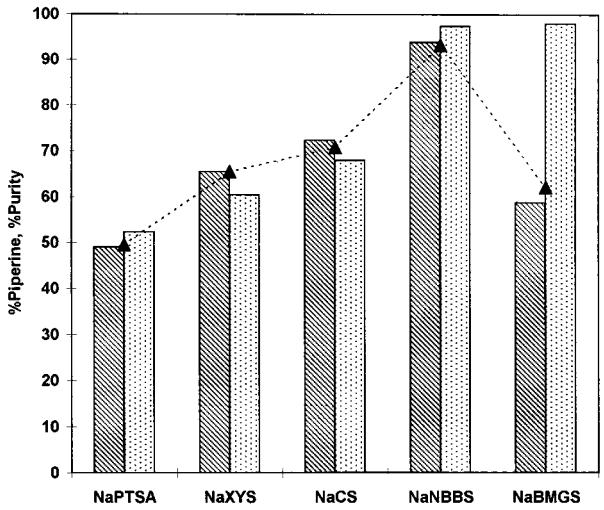


Figure 4. Comparison of extraction efficiency of hydrotropes for piperine (temperature = 300 K, concentration = 2 mol/dm^3, period of extraction = 2.0 h): ▲, expected % piperine extracted; striped bar, actual % piperine extracted; dotted bar, % purity.

be estimated from its effective carbon chain lengths (n_c)[25]

$$v = (27.4 + 26.9n_c) \quad (1)$$

where n_c represents the number of carbon atoms that can enter into the formation of the hydrophobic space analogous to the hydrophobic core of surfactant micelles, usually ignoring one carbon close to the polar group. The shape of the hydrophobic region of the hydrotrope aggregates is not conclusively known, but a dissolved solute does experience a reduced micropolarity and increased microviscosity in hydrotrope solutions.[13] The hydrophobic region of the hydrotrope aggregates seems to be accommodating the dissolved solutes. These hydrophobic volumes were estimated to be 215.7 Å^3 for NaNBBS, 188.8 Å^3 for NaCS, 161.9 Å^3 for NaXS, and 135 Å^3 for NaPTS.

The increased solubilization with increasing hydrophobic volume indicates that hydrotropic solubilization

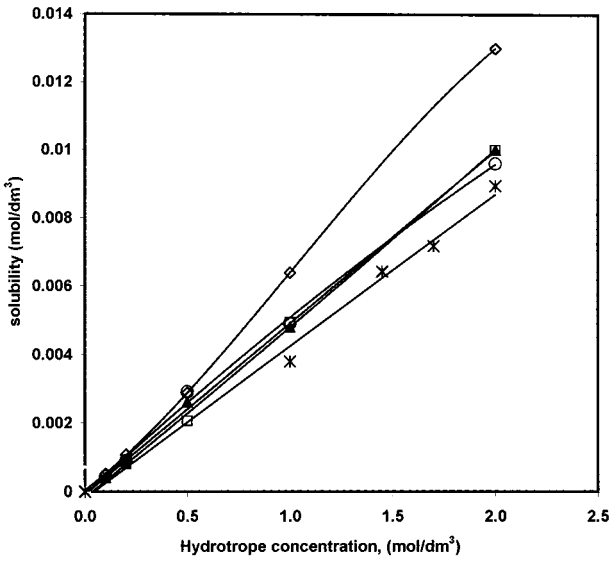


Figure 5. Solubilty of piperine in different hydrotropes (temperature = 300 K): ◇, NaNBBS; ▲, NaCS; □, NaPTSA; ○, NaXS; *, NaBMGS.

could be a consequence of the hydrophobic domains present within the hydrotrope aggregates, which can provide a microenvironment compatible with the hydrophobic nature of piperine. Piperine is nearly water-insoluble otherwise. It is also possible that the aggregation behavior of the hydrotrope itself is further promoted by the presence of a very hydrophobic solute. The solubilization is then analogous to co-aggregation of the hydrotrope molecules and the solute, giving rise to selectivity in hydrotropic solubilization. If the solute intercalates easily between the hydrotrope molecules in the aggregates, then its solubilization should be better. Because the hydrophobicity of the aromatic sulfonates increases with increasing alkyl group length, they display an increasing tendency for the solubilization of nonpolar molecules.[26]

NaBMGS is a short-chain aliphatic sulfate that is structurally very different from the conventional aromatic sulfonates but that still shows a very high selectivity toward piperine (Figure 4). Hydrotropic extraction is a phenomenon, as discussed earlier, that probably involves adsorption of hydrotrope on plant cells, penetration into the cellular matrix, and then solubilization of the active component. The surface tension values of hydrotrope solutions are 37 dyn/cm for NaBMGS, 50−53 dyn/cm for NaPTS and NaXS, 43 dyn/cm for NaCS, and 40.3 dyn/cm for NaNBBS at their minimum hydrotrope concentrations.[13] NaBMGS shows the least surface tension, but the decrease is gradual over a large concentration range, and the molecules occupy larger areas at the interface than aromatic sulfonates, probably because of the gauche conformation at the butyl group joining the glycol part or the flat orientation of the molecule at the interface.[13] For the penetration of a hydrotrope into the biomatrix, a lower surface tension is useful in overcoming the surface capillary forces within the cellular surface.[27] In this regard, NaNBBS and NaBMGS have excellent wettability characteristics as compared to NaXS and NaPTS.

Figure 5 shows the solubility of piperine in different hydrotrope solutions. In water, piperine is soluble at a 1.4×10^{-5} mol/dm^3 concentration. In 0.5 mol/dm^3 NaNBBS solutions, the piperine solubility increases by nearly 230 times to 3.221×10^{-3} mol/dm^3. Only above the MHC of NaNBBS (0.1 mol/dm^3) is the increase in the solubility significant. Similar solubility behavior is evident in other hydrotrope solutions.

The solubility of a solute in a hydrotrope solution (S) is usually correlated by an exponential relation, in a manner analogous to salting-out, to the hydrotrope concentration (C_t) and the solute solubility in water (S_w)

$$\log(S/S_w) = K_s C_t \tag{2}$$

where the Setchnow constant (K_s) represents the efficiency of a hydrotrope. This expression, however, cannot represent the saturation limits observed in hydrotrope solutions. An association model was recently proposed for hydrotrope solubilization that considers aggregation of the hydrotrope molecules in a stepwise manner and then solubilization as the co-aggregation of a solute with these aggregates.[28] The total concentration of hydrotrope (C_t) is related to the hydrotrope monomer concentration (H_1) through eq 3 under the assumption that the aggregation constant decreases with increasing aggregation number (n) as $K_n = K_2/n$, where K_2 is the dimerization constant for hydrotrope molecules.

$$C_t = H_1[2 \exp(K_2 H_1) - 1] \tag{3}$$

The increased solubility of a solute (ΔS) due to the association of solute molecules with the hydrotrope aggregates can then be correlated with the hydrotrope concentration by

$$\Delta S = 2(K_s/K_2)[S_w]\{\exp(K_2 H_1) - (1 + K_2 H_1)\} \tag{4}$$

where the constant K_s characterizes the interaction between the hydrotrope aggregates and the solute molecules. A higher value of K_s signifies a stronger interaction of the solute with the hydrotrope aggregates.

Although the association model is an approximation of the actual aggregation process of a hydrotrope and subsequent solute solubilization, it explicitly considers hydrotropy as an aggregative phenomenon. In the present case, the association of piperine with hydrotrope aggregates is reflected in the values of K_s, which represent the strengths of the interactions of piperine with different hydrotropes. The solubility data were fitted to the model eqs 3 and 4 to estimate K_s values for the different hydrotropes. The K_s values found are 368 dm^3/mol, the highest, for NaNBBS; 320.9 dm^3/mol for NaCS; 301.7 dm^3/mol for NaXS 281.3 dm^3/mol for NaPTS; and 232.4 dm^3/mol for NaBMGS. The hydrotrope−hydrotrope association constants (K_2), however, had very low values of 0.101, 0.11, 0.11, 0.06, and 0.09 dm^3/mol for NaNBBS, NaCS, NaXS, NaPTS, and NaBMGS, respectively. The highly ionic nature of the hydrotrope would not permit a close packing of these molecules, as is evident from the dimerization constant values. Instead, the presence of piperine might augment the hydrotrope association, as the process is akin to co-aggregation.

The expected extraction of piperine was estimated on the basis of the saturation solubilities of piperine in different aromatic sulfonate solutions at 2.0 mol/dm^3 concentration. There was very little deviation in the expected values of extraction from the actual experimental values for the given set of conditions (Figure 4).

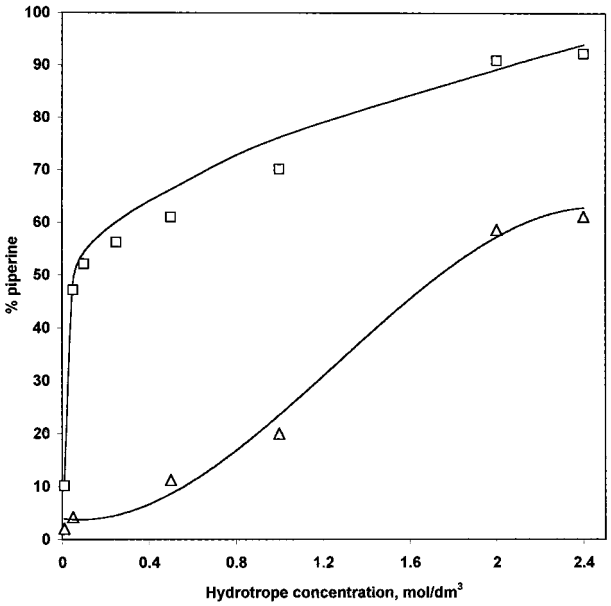


Figure 6. (—) Expected and (points) actual extraction of piperine with aqueous NaBMGS and NaNBBS solutions (temperature = 300 K, time = 2.0 h): □, 2.0 mol/dm^3 NaNBBS; ▲, 1.7mol/dm^3 NaBMGS.

Extraction of Piperine with Hydrotrope Solutions (NaBMGS and NaNBBS). Figure 6 shows the effect of the NaBMGS concentration on the extraction of piperine from *P. nigrum* fruits. Above the minimum hydrotrope concentration of NaBMGS,[13] i.e., 0.8 mol/dm^3, as the concentration of NaBMGS was increased from 1.0 to 2.5 mol/dm^3, the percentage extraction of piperine increased from 20 to 60%. Because hydrotropy is mainly operative above the minimum hydrotrope concentration, the extraction of piperine rose markedly at concentrations above the MHC of the hydrotrope and then leveled off at higher hydrotrope concentrations. Below the MHC, however, the increase was marginal. At a hydrotrope concentration of 1.7 mol/dm^3, the solubility of piperine in the aqueous hydrotrope phase was 5.6×10^{-3} mol/dm^3, an increase by a factor of 400 over its water solubility. The purity of piperine, however, decreased slightly when the hydrotrope concentration was increased to 2.0 mol/dm^3 and above.

NaNBBS has an MHC of 0.1 mol/dm^3. The extraction experiments were, therefore, conducted at concentrations just below and well above this value. As the NBBS concentration was increased from 0.05 to 2.4 mol/dm^3, the amount of piperine recovered by precipitation from the extract solution by dilution with water also increased from 57 to 95% and remained constant thereafter (Figure 6). The diluted hydrotrope solution retained in each case the remaining amount of piperine, as the extraction from the raw material was complete. The recovered piperine amounts are clearly indicative of a highly efficient extraction by NaNBBS solutions. No significant decrease in the purity was observed with the increase in hydrotrope concentration, which remained constant at 92−93%. Figure 6 also compares the actual extraction of piperine with the expected extraction as determined from the solubility of piperine in NaNBBS solutions. The actual recovery is slightly lower than the expected extraction of piperine. This slight reduction in the recovery could be due to a small amount of piperine remaining in the solid cake. The optimum concentration of NaNBBS required for extraction with optimum recovery and purity was found to be 2.0 mol/dm^3 as no significant increase in piperine extraction was observed at a 2.4 mol/dm^3 hydrotrope concentration. The solubility data also indicated that NBBS was the most effective hydrotrope in dissolving piperine. Even at concentrations as low as 0.5 mol/dm^3, almost 70% extraction of piperine could be achieved in 2 h.

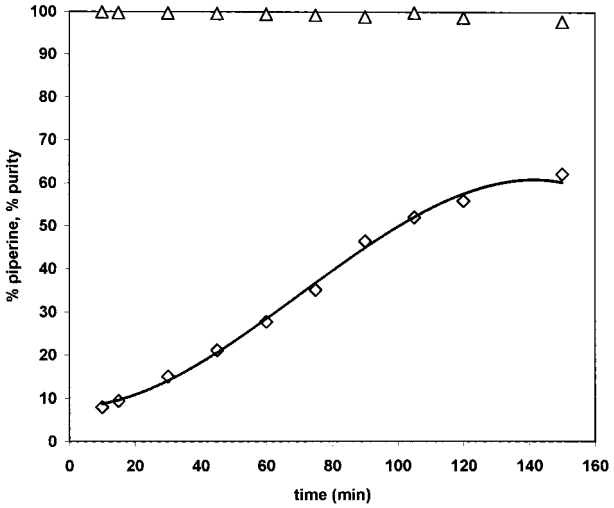


Figure 7. Extraction of piperine with different concentrations of aqueous NaBMGS solution (temperature = 300 K, time = 2.0 h, solid loading = 10% w/v, speed of agitation = 1100 rpm): ◇, % piperine extracted; △, % purity.

At lower concentrations, close to the MHC, some hydrotrope monomers can still become incorporated into the cell wall/membrane structure, which can cause the membranes to lose their integrity.[29] At concentrations much above the MHC, the solubilization capacity of the hydrotrope aggregates is much higher, i.e., almost as an exponential function of the hydrotrope concentration. As the solubilization within the cell matrix is easier and more rapid, the transport of piperine back into the external liquid solution should be faster, and its purity should also possibly be better, as other oleoresins dissolve less readily into hydrotrope solutions in the same amount of time.

Figure 7 shows that, for 1.7 mol/dm^3 NaBMGS solution, the percentage extraction of piperine increased from 10% in the first 15 min to 59% after 2.0 h and remained constant thereafter. The sigmoidal nature of the extraction curve indicates that the extraction is a second-order process, i.e., initially, the rate of extraction is slow, followed by a faster extraction stage before a plateau of limiting value is reached. The final extraction limit is determined by the solubility limit of piperine in the hydrotrope solution under given conditions. It appears that the extraction needs to overcome two resistances in the process. One might have to consider then the cellular structure of the complex biomatrix to understand these resistances. The ordered structure of the cells would resist the penetration of hydrotrope molecules first into the cellulosic layer and then further into the phospholipid bilayer before it can access the piperine dispersed through cell.

The rate of extraction should depend on the ease with which the hydrotrope can penetrate into the biomatrix and also on the solubility of piperine in the hydrotrope solution. In the case of NaNBBS at a concentration of 0.05 mol/dm^3, below its MHC, 45% piperine was extracted within 10 min, and the extraction remained fairly constant even after 2 h, with only a slight increase to 57%. At higher NaNBBS concentrations, however, a

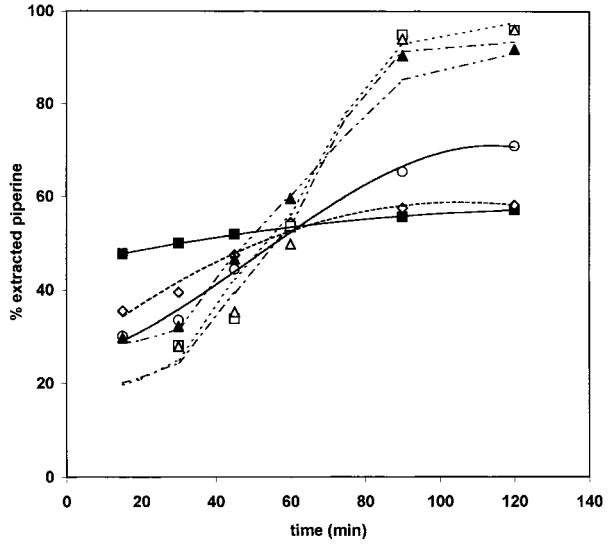


Figure 8. Kinetics of extraction of piperine with different concentrations of NaNBBS (temperature = 300 K, solid loading = 10% w/v, speed of agitation = 1100 rpm): ■, 0.05; ◊, 0.5; ○, 1.0; ▲, 2.0; □, 3.0; △, 3.4 mol/dm^3.

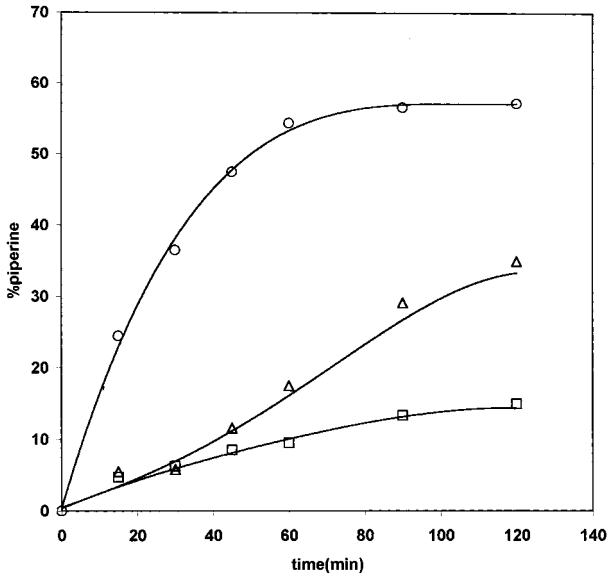


Figure 9. Extraction of piperine with surfactants SLS and CTAB (concentration = 0.5 mol/dm^3, temperature = 300 K, solid loading = 10% w/v, speed of agitation = 1100 rpm): □, SLS; △, CTAB; ○, NaNBBS.

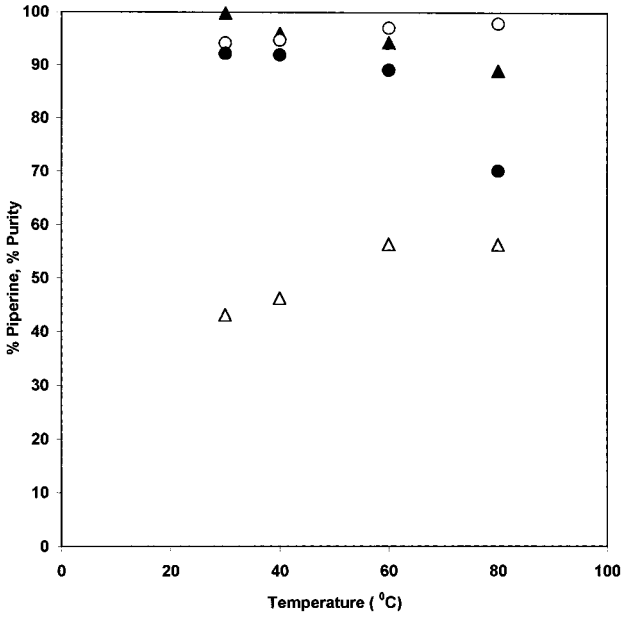


Figure 10. Extraction of piperine using NaBMGS and NaNBBS at different temperatures (concentration = 2.0 mol/dm^3, time = 2.0 h): △, % piperine extracted (NaBMGS); ▲, % purity piperine (NaBMGS); ○, % piperine extracted (NaNBBS); ●, % purity piperine (NaNBBS).

lag time of 30 min was observed, with just 25% extraction of piperine. Rapid extraction of piperine was achieved in the next 1 h. Finally, after 2 h, the extraction reached 95% (Figure 8). The rate of extraction is determined by penetrability, which depends on the hydrotrope monomer concentration and the solubility of piperine in the hydrotrope aggregates. At hydrotrope concentrations of 0.05 and 0.5 mol/dm^3, the percentage extraction followed a rectangular shape with a finite initial rate, which is typical of a first-order process. Because hydrotrope is present predominantly in monomeric form at these concentrations, its adsorption on the solid surface is preferential, which also leads to a higher penetration rate. At the higher hydrotrope concentrations of 1.0 and 2.0 mol/dm^3, however, the extraction showed a sigmoidal behavior with almost zero initial slope, typical of a second-order process. The initial resistance to solubilization probably arises because few hydrotrope molecules are present as monomers rather than aggregates, which avoids adsorption on the solid surface. Once the penetration takes place, however, solubilization is faster.

With the increased concentration of NaNBBS, a larger osmotic pressure also develops across the cell wall. Thus, at higher concentrations, hydrotrope solution enters the cell matrix relatively slowly. After the initial lag period, during which the hydrotrope monomers penetrate into the cell wall and destabilize the liquid-crystalline nature of the bilayer, the turgidity of the cell wall is lost. Further penetration of aqueous solution into the cellular matrix and transport of piperine out to the external hydrotrope medium are then rapid steps.

Figure 9 shows a comparison of the extraction profiles of NaNBBS with the cationic surfactant cetyl trimethylammonium bromide (CTAB) and the anionic surfactant sodium lauryl sulfate (SLS) at the same concentration. At a concentration of 0.5 mol/dm^3, SLS showed a sigmoidal extraction pattern, but after 2 h, only 15% extraction of piperine was observed. CTAB, on the other hand, gave a somewhat better extraction of 35% within 2 h. In comparison to these surfactants, NaNBBS demonstrated faster and better hydrotropic extraction. The surfactants CTAB and SLS induced permeability in the cell wall but did not promote the complete extraction of piperine.

Hence, it is proposed that hydrotropic extraction proceeds in two steps: first, penetration of hydrotrope molecules into external cell wall, which offers a major resistance to mass transfer of solution into the cell, and second, solubilization of piperine and its back transfer to the external solution. The initially observed extraction could result from piperine being directly exposed to the hydrotrope solution upon comminution of the raw materials.

Figure 10 shows the actual recovery of piperine from NaBMGS solutions, which increased from 43 to 56%, with a significant decrease in the purity, when the extraction was conducted at elevated temperatures for

Table 1. Effect of Particle Size on the Extraction of Piperine[a]

particle size (μm)	% piperine	% purity
53	90.14	98.3
180	92.14	97.3
600	95.12	90.2
710	96.15	89.12

[a] Concentration = 2.0 mol/dm^3, temperature = 300 K, time = 2.0 h, solid loading = 10% w/v.

the same period. Figure 10 also shows the effect of temperature, in the range 30−65 °C, on the degree of extraction of piperine using NaNBBS solutions. There was a very slight increase in the percentage extraction in 2 h, but the purity of the extract decreased substantially from 92 to 70%. It is preferable, therefore, to conduct the extraction at ambient conditions. At higher temperatures, more lysis of the cell structure might take place, and the cell wall might become more permeable to the hydrotrope solution. Because of both the breakdown and the solubilization of the cellulose polymers within the cell wall, the contribution of the polymers to the firmness of the cell wall could be reduced. The enhanced cell rupture at elevated temperatures also facilitates diffusion of undesirable oleoresins into the external bulk hydrotrope solution, resulting in decreased selectivity. The decrease in purity at higher temperatures could also be due to increased solubilization of other oleoresins from the cells by the hydrotrope solution. At a temperature of 30° C, probably only destabilization of the liquid lamellar structure of cell membrane takes place, which enables more selective transport of piperine into hydrotrope solutions.

Beyond the critical speed of agitation, the major resistance to mass transfer lies within the particle. To substantiate our results on intraparticle resistance, the particle size of the raw material was varied from 50 μm to a mesh size of 710 μm (Table 1). A significant decrease in the purity of the extracted piperine from 98 to 89% was observed on reduction of the particle size. The increased rupturing of the cell walls upon size reduction increased the accessibility of the hydrotrope solution to the cellular matrix. This might lead to solubilization of other solutes along with piperine. Fine grinding is expensive, but it provides more rapid and possibly thorough leaching. However, it suffers the disadvantage that the weight of the liquid associated with the settled solid might be high, so a considerable amount of solvent is used to wash the solid retentate/cake free of solute, making the resulting solution quite dilute. Coarsely ground particles, on the other hand, leach more slowly and possibly less thoroughly, but on draining, they retain relatively little solution, require less washing, and thus provide a more concentrated final solution.

Conclusion

Hydrotrope solutions permeabilized the pericarp of *Piper nigrum* fruits and, therefore, facilitated the selective extraction of piperine. The hydrotrope molecules probably adsorb on the cellulosic cell wall, disorganize its structure, and then penetrate into the cell membrane, assisting in disordering the amphiphilic lipid bilayer and permeabilizing it to enable the easy release of piperine. The presence of phosphorus reducing sugars and amino acids in the extract phase suggests the breakdown of cellulose, as well as membrane proteins, to some extent. A substantial enhancement in extraction rate was observed as a result of the structural changes in the biomatrix triggered by the hydrotrope action. The primary step of the extraction process involved penetration of the hydrotrope into the external cellulosic cell walls, which offer major resistance to mass transfer of the hydrotrope solution. The second step constitutes the solubilization of piperine and its back transfer into the external solution. The process was optimized with respect to concentration, temperature, and particle size required for extraction of piperine. In addition, piperine could be recovered by simple dilution with water in highly pure form and could be directly applied for formulations as it was free from any contamination. Hydrotropic extraction shows tremendous potential for cell permeabilization and selective extraction of bioactive compounds on a commercial scale. Although we have hypothesised the mechanism of hydrotropic extraction in terms of destabilization of the cellular wall structure, further investigations are needed to confirm this theory.

Acknowledgment

We acknowledge financial support for this work to the Council for Scientific and Industrial Research (CSIR), Government of India.

Literature Cited

(1) Shreiber, W. L.; Scharpf, L. G.; Katz, I. Flavors and Fragrances: The Chemistry Challenges. *CHEMTECH* **1997**, 58−61.

(2) Trease, G. E.; Evans, W. C. *Pharmacognosy*; Baillere Tindall: London, 1983; p 570.

(3) Reen, R.; Rashmet, K.; J. Potent chemoprotective effects against procarcinogens. *Ethanopharmacology* **1997**, *58* (3), 165−173; cf. *Chem. Abstr.* **1997**, *128*, 110828v.

(4) Timmers, L. *Herbal Medicines Used against Epilepsy in Developing Countries*; Publication Number PUG/94-4; Publicaties Wetenschapswinkel Geneesmiddelen: Vrouwen, The Netherlands, 1994.

(5) Karan, R. S, Bhargava, V. K., Garg, S. K. Effect of piperine on the pharmacokinetic profile of isoniazid in rabbits. *Indian J. Pharmacol.* **1988**, *30*, 254−256.

(6) Sharma, A.; Gautam, S.; Jadhav, S. S. Spice extracts as dose modifying factors in radiation inactivation of bacteria. *J. Agric. Food. Chem.* **2000**, *48* (4), 1340−1344.

(7) Marion, L. The Pyrrolidine Alkaloids. In *The Alkaloids Chemistry and Physiology*; Manske, R. H. F., Holmes, H. L., Eds.; Academic Press: London, 1960; Vol. I, p 168.

(8) Vidal, J. P.; Richard, H. Production of black pepper oleoresin by dense carbondioxide or carbondioxide ethanol extraction. *Sci. Aliments* **1987**, *7* (3) 481−498; cf. *Chem. Abstr.* **1987**, *107*, 19661t.

(9) Hans, J. Extraction of organic constituents from solids. German Patent DE 28/02/1987, 1987; cf. *Chem. Abstr.* **1987**, *109*, P172901e.

(10) Rastogi, N. K.; Niranjan, K. Enhanced Mass Transfer during Osmotic Dehydration of High-Pressure Treated Pineapple. *J. Food Sci.* **1998**, *63* (3), 508−511.

(11) Qin, W.; Tao, Z.; Younhui, Y.; Youyuan, D. Intensification of Curcumin Leaching with Ultrasound. *Value Adding, Solvent Extr.* **1996**, *2*, 1679−1684.

(12) Gaikar, V.G.; Sharma, M. M. Separations with Hydrotropes. *Sep. Technol.* **1993**, *3*, 3−11.

(13) Balasubramanian, D.; Srinivas, V.; Gaikar, V. G.; Sharma, M. M. Aggregation Behavior of Hydrotropic Compounds in Aqueous Solution. *J. Phys. Chem.* **1989**, *93*, 3865−3870.

(14) Bhat. M.; Gaikar. V. G. Characterization of Interaction between Butyl Benzene Sulfonates and Cetyl Trimethylammonium Bromide in Mixed Aggregate Systems. *Langmuir* **1999**, *15*, 4740−4751.

(15) Verzele, M.; Mussche, P.; Gureshi, S. A. High performance liquid chromatographic analysis of the pungent principles of pepper and pepper extracts. *J. Chromatogr.* **1979**, *72*, 493−497.

(16) Fiske, C. H.; Subbarow, Y. The Colorimetric Determination of Phosphorus. *J. Biol. Chem*. **1925**, *66*, 375−400.

(17) Jayaraman, J. *Laboratory Manual in Biochemistry, Biomolecules I: Carbohydrates*; New Age International Ltd.: New Dehli, India, 1996; p 51.

(18) Touchstone, J. C. *Practice of Thin Layer Chromatography*; John Wiley and Sons: New York, 1992; p 153.

(19) Felix, H. Permeabilised Cells. *Anal. Biochem*. **1982**, *120*, 211−234.

(20) Nixdroff, B. Interaction of LPS with Detergents and Its Possible Role in the Detergent Resistance of the Outer Membrane of Gram-Negative Bacteria. *Biochim. Biophys. Acta* **1978**, *510*, 87−98.

(21) Rosen, M. J.; Fei, L.; Zhu, Y.; Morrall, S. The Relationship of the Environmental Effect of Surfactants to Their Interfacial Properties. *J. Surfactants Deterg.* **1999**, *2* (3), 343−347.

(22) Arnot, H. J. *Scanning Electron Microscopy Of Black Pepper (Piper nigrum) Fruits*; Department of Biology and Center for Electron Microscopy, The University of Texas at Arlington: Arlington, TX, 2000.

(23) Steudle, E. The biophysics of plant water: Compartmentation, coupling with metabolic processes, and water flow in plant roots. In *Water and Life: A Comparative Analysis of Water Relationships at the Organismic, Cellular, and Molecular Levels*; Somero, G. N., Osmond, C. B., Bolis, C. L., Eds.; Springer-Verlag: Berlin, 1992; pp 173−204.

(24) Srinivas, V.; Rodley, A.; Ravikumar. K; Robinson, W. T.; Turnbull, M. M.; Balasubramanian, D. Molecular Organization in Hydrotrope Assemblies. *Langmuir* **1997,** *13*, 3235−3239.

(25) Tanford, C. Theory of micelle formation in aqueous solutions. *J. Phys. Chem*. **1974**, *78* (24), 2469−2479.

(26) Srinivas, V.; Sundaram, C. S.; Balasubramanian, D. Molecular structure as a determinant of hydrotropic action: A study of polyhydroxy-benzenes. *Indian J. Chem*. **1991**, *30B*, 147−152.

(27) Tadros, T. F. *Surfactants in Agrochemicals*; Surfactant Science Series; Marcel Dekker: New York, 1995; p 54.

(28) Gaikar, V. G.; Phatak, P. V. Selective Solubilisation of Isomers in Hydrotrope Solutions: o−p/-Chlorobenzoic acids and o−p/-Nitroanilines, *Sep. Sci. Technol*. **1999**, 2716.

(29) Wu, J.; Ruan, Q.; Lam, P. Effects of Surface Active Medium Additives on Insect Cell Surface Hydrophobicity Relating to Cell protection against Bubble Damage. *Enzyme Microbiol. Technol*. **1997**, *21*, 341−348.

Received for review September 21, 2001
Revised manuscript received March 19, 2002
Accepted March 24, 2002

IE0107845