Asian Journal of Chemistry; Vol. 25, No. 1 (2013), 231-236

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.12915

Extraction of Vanillin Through Hydrotropy

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(*Received*: 22 October 2011; *Accepted*: 20 July 2012) AJC-11853

Natural vanillin (4-hydroxy-3-methoxybenzaldehyde) produced from vanilla beans and other naturals is one of the most common flavour chemicals widely used in a broad range of flavours. In present work,vanilla beans are permeabilized by aqueous hydrotrope solutions followed by extraction and solubilization of vanillin into the hydrotrope solutions of nicotinamide, sodium salicylate, resorcinol and citric acid. The influence of a wide range of hydrotrope concentrations (0-3.0 mol/L) and different system temperatures (303-333 K) on the solubility of vanillin has been studied. The solubility of vanillin increases with increase in hydrotrope concentration and also with system temperature and nicotinamide were found to be effective for the selective extraction of vanillin with a recovery of 85 % from the aqueous solution of hydrotropes with high purity. The process was further optimized with respect to concentration of hydrotropes and temperature of extraction. A minimum hydrotrope concentration was found essential to show a significant increase in the solubility for vanillin + water system. Consequent to the increase in the solubility of vanillin, the mass transfer coefficient was also found to increase with increase in hydrotrope concentration at 303 K. The enhancement factor, which is the ratio of the value in the presence and absence of a hydrotrope, is reported for both solubility and mass transfer coefficient of vanillin. The Setschenow constant, K_s, a measure of the effectiveness of a hydrotrope, was determined for each case.

Key Words: Hydrotrope, Solubility, Extraction, Mass transfer coefficient, Vanillin.

INTRODUCTION

Hydrotropy is one of the potentially attractive techniques that can be considered to separate close boiling point isomeric / nonisomeric mixtures. The phenomenon of hydrotropy was first reported by Neuberg in 1916[1] to solubilize sparingly soluble compounds. Winsor[2] considered hydrotropy as a solubilization phenomenon while Ueda[3-5] proposed the formation of molecular complexes at low hydrotrope concentrations. Hydrotropes are a class of highly water soluble salts/molecules that are characterized by an amphiphilic molecular structure with an ability to dramatically increase the solubility of sparingly soluble organic compounds in water, often by several orders of magnitude[6]. Besides the contamination of the product by hydrotrope is minimal and can be reduced to below an acceptable level, if any, simply by washing with water[7]. Hence extraction of vanillin from vanilla bean is much preferable through hydrotropy.

The concentration at which self association begins is denoted as the minimum hydrotrope concentration (MHC) and is often indicated by changes in solution properties such as density, conductivity and surface tension[8]. The formation and nature of aggregates of hydrotrope molecules in water is essential to make a strong association between microscopic assembly and the corresponding macroscopic behaviour. This hydrotropic phenomenon can be adapted to industrial scale[9,10].

Hydrotropic solubilization has been claimed to be a collective molecular phenomenon, possibly occurring by the aggregation of a solute with the hydrotrope aggregates and the self-aggregation of hydrotrope molecules in aqueous solutions is considered to be a prerequisite for the enhanced solubility of the solute and drug solubilization[11-13]. A hydrotrope above minimum hydrotrope concentration is expected to form organized loose nanoassemblies with distinct hydrophobic regions where the solute can be solubilized[14-16]. The solute molecules may also take part in the aggregation process of the hydrotrope, thereby forming coaggregates with the hydrotrope molecules in aqueous solutions. The formation of a stable coaggregate depends on the molecular structure as well as the functional group(s) attached to the carbon skeleton of the solute as it would govern the intercalation of the solute between the hydrotrope molecules. The solubilization of a solute is influenced by its hydrophobic part and also the chain length of an alkyl group of a hydrotrope[17-23].

Vanillin is widely used flavour in food, perfume, beverages and pharmaceutical industries[24,25]. The increasing consumer request for natural products created many biotechnological processes to produce 'natural vanillin'[26,27]. Microbial transformation from natural substrates, including phenolic stibenes[28], lignin[29], isoeugenol[30], eugenol[31],vanillic acid[32,33]. Extraction of vanillin can also be done by percolation method, oleoresin method and supercritical fluid extraction method.

The mass transfer coefficient was found to be very low solely due to the poor solubility of the hydrophobic reactant in the aqueous phase. The ice cream and chocolate industries together comprise 75 % of the market for vanillin as a flavour, with smaller amounts being used in confectionaries and baked goods. Hence, it has become essential to enhance the solubility and mass transfer coefficient of vanillin to get the maximum rate of output of the desired products made from vanillin. All hydrotropes are non-reactive, non-toxic and do not produce any temperature effect when dissolved in water. The low cost and easy availability are other factors considered in the selection of hydrotropes.

EXPERIMENTAL

All the chemicals used in this work were manufactured by the Loba Chemie Pvt. Ltd., Mumbai. with a manufacturer's stated purity of 99.9 %. The hydrotropes used in this work *viz.*, nicotinamide, sodium salicylate, resorcinol and citric acid are of AnalaR grade. Double distilled water was used for the preparation of hydrotropic solutions.

Methods

Vanilla bean was first pulverized to a coarse powder and then separated into batches of different sizes using mechanical sieves. The agitation experiments were carried out in a fully baffled cylindrical glass vessel equipped with a 6 bladed turbine impeller. This entire assembly was kept in a constant-temperature bath. A 10 g sample of ground vanilla bean was added to 250 mL of 0.1 mol/L of hydrotrope solution of a known concentration in the range 0.05-3 mol/L in the glass vessel. The suspension was agitated at 1100 rpm for a period of 2 h at 300 K. The solution was then allowed to settle for 1 h and was subsequently filtered under vacuum within 10 min. The filtrate was then diluted with water at 300 K to bring the hydrotrope concentration below its minimum hydrotrope concentration.

Vanillin is precipitated from the solution as fine crystals over a period of 1 h. The suspension was then centrifuged to separate the solid product from the remaining solution. The precipitate was dried and analyzed for purity using HPLC.

The thermostatic bath method used for the determination of solubility values consisted of a thermostatic bath and a separating funnel. Measurement of the solubility of vanillin was carried out at 303, 313, 323 and 333 K. For each solubility test, an excess amount of powdered solid was placed in a separating funnel and a solution of the hydrotrope of known concentration was added. The separating funnel was sealed to avoid evaporation of the solvent at higher temperatures. The hydrotrope solutions of different concentrations were prepared by dilution with distilled water. The separating funnel was immersed in a constant temperature bath fitted with a temperature controller that could control the temperature within ± 0.1 ºC. The setup was kept overnight for equilibration. After equilibrium was attained, the solution was filtered from the remaining solid. The concentration of the dissolved organic compound was analyzed using HPLC (Manufacturer - ESA, Dionex; Type - UltiMate 3000) with the following conditions: LC mode, reverse phase chromatography; Packing material, silica-C18; mobile phase, water/acetonitrile (CH_3CN)/acetic acid (10 %); Interaction, hydrophobic. All the solubility experiments were conducted in duplicate to check their reproducibility. The observed error was < 2 %.

The experimental setup for the determination of the mass transfer coefficient consisted of a vessel provided with baffles and a turbine impeller run by a motor to agitate the mixture. The speed of the impeller at 600 rpm was selected to achieve effective mixing and was maintained at the same value for all experiments. The vessel used for mass transfer studies had a height of 40 cm and an inner diameter of 15 cm. The turbine impeller had a diameter of 5 cm, width of 1 cm and length of 1.2 cm. It had four blades and rotated at 600 rpm. The baffle has a height of 40 cm, diameter of 1.5 cm and four baffles arranged at 90º to each other. For each run, to measure the mass transfer coefficient, an excess amount of powdered solid was added to the aqueous solution of the hydrotrope of known concentration. The sample was then agitated for 600, 1200, 1800 or 2400 s and the mixture was transferred to a separating funnel[15-17]. After allowing the sample to stand for some time, the solution was filtered from the remaining solid. The concentration of the solubilized organic compound vanillin in aqueous hydrotrope solutions at time t was analyzed in the same way as done for solubility determinations using HPLC.

RESULTS AND DISCUSSION

Solubility: Extracted vanillin has been shown in a schematic comparative HPLC chromatogram in Fig. 1. Experimental data on the effect of hydrotropes on the solubility of vanillin at different temperatures are plotted (Figs. 2-5). Nicotinamide is one of the hydrotropes used in this study. The solubility of vanillin in water at 303 K in the absence of any hydrotrope is 6.71×10^{-2} mol/L (Fig. 2). Solubility of vanillin did not shown any appreciable increase even after the addition of hydrotrope in the aqueous phase but on subsequent increase in the concentration of hydrotrope, the solubility of the vanillin in aqueous phase increases significantly. This concentration *i.e.* 0.20 mol/ L of hydrotrope required to induce a significant increase in the solubility of vanillin in water is termed as minimum hydrotrope concentration (MHC). The solubility of vanillin in water did not show any appreciable increase even after the addition of 0.20 mol/L of nicotinamide to the aqueous solution.

Therefore, it appears that hydrotropic solubilization is evident only above the MHC, irrespective of the system temperature. With respect to each solute, hydrotropy does not seem to be operative below the MHC, which may be a characteristic of a particular hydrotrope. This MHC value assumes greater significance in the context of recovery of hydrotrope solutions. Since hydrotropy appears to operate only at significant concentrations of hydrotrope in water, most hydrotropic solutions release the dissolved vanillin on dilution with water below the MHC. The knowledge of MHC values is necessary especially at industrial levels, as it ensures ready recovery of the hydrotrope for re-use.

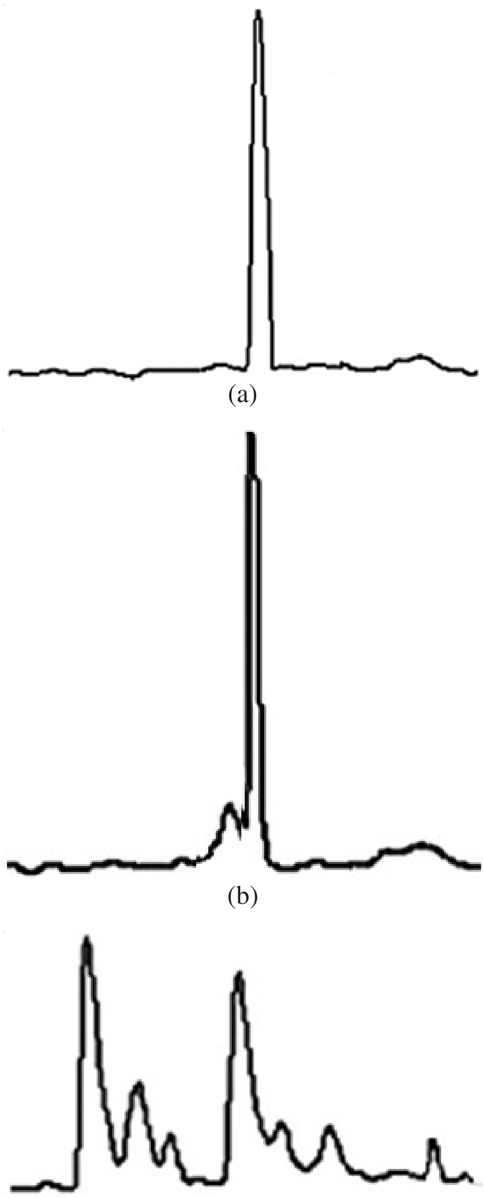

Fig. 1. Comparative HPLC chromatogram of extracted vanillin (a) Pure vanillin, (b) Nicotinmide extracted vanillin (c) Soxhlet extracred vanillin

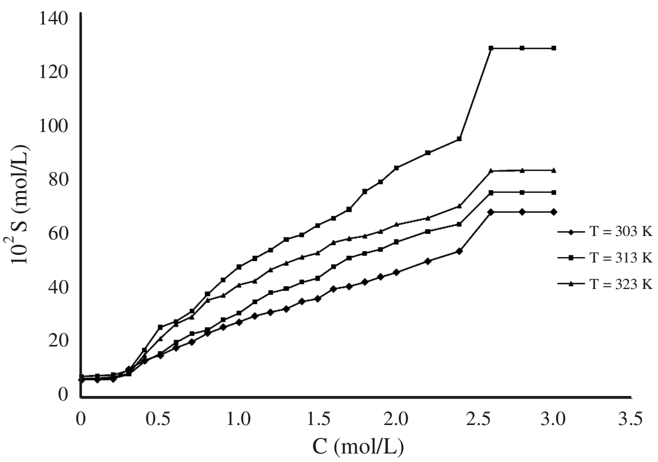

Fig. 2. Effect of nicotinamide concentration (C) on the solubility (S) of vanillin in water at different temperatures

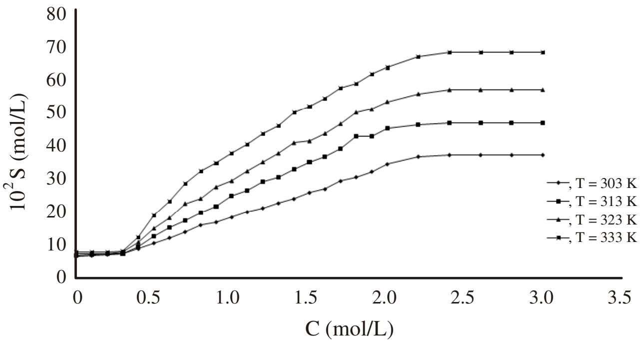

Fig. 3. Effect of sodium salicylate concentration (C) on the solubility (S) of vanillin in water at different temperatures

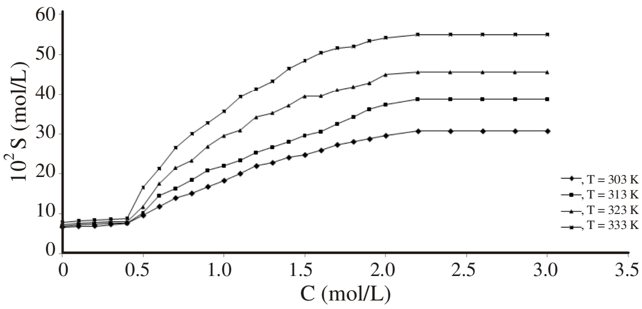

Fig. 4. Effect of resorcinal concentration (C) on the solubility (S) of vanillin in water at different temperatures

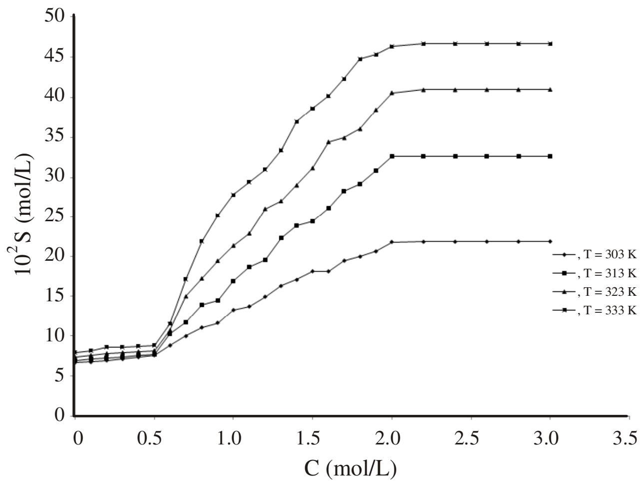

Fig. 5. Effect of citric acid concentration (C) on the solubility (S) of vanillin in water at different temperatures

The solubilization effect varies with the concentration of hydrotrope (Fig. 2). In the present case, a clear increasing trend in the solubility of vanillin was observed above the MHC of nicotinamide. This increasing trend was maintained only up to a certain concentration of nicotinamide in the aqueous solution, beyond which there is no appreciable increase in the solubility of vanillin. This concentration of nicotinamide (hydrotrope) in the aqueous solution is referred to as the maximum hydrotrope concentration (C_{max}) which is 2.60 mol/L for nicotinamide. C_{max} values of sodium salicylate, resorcinol and citric acid with respect to vanillin are 2.40, 2.20 and 2.00 mol/L, respectively (Table-1). From the analysis of the experimental data, it was observed that a further increase in the hydrotrope concentration beyond C_{max} did not bring any appreciable increase in the solubility of vanillin even at concentrations of up to 3 mol/L of nicotinamide in the aqueous

TABLE-1 MINIMUM HYDROTROPE CONCENTRATION (MHC) AND C_{max} VALUES OF HYDROTROPES		
Hydrotropes	MHC (mol/L)	C_{max} (mol/L)
Nicotinamide	0.20 ± 0.004	2.60 ± 0.052
Sodium salicylate	0.30 ± 0.005	2.40 ± 0.045
Resorcinol	0.40 ± 0.007	2.20 ± 0.032
Citric acid	0.50 ± 0.008	2.00 ± 0.038

solution. Similar to MHC values, the C_{max} values of hydrotropes remained unaltered with an increase in system temperature.

The knowledge of MHC and C_{max} values of each hydrotrope with respect to a particular solute assumes greater significance in this study, since it indicates the beginning and saturation of the solubilization effect of hydrotropes. The values of MHC and C_{max} of a hydrotrope with respect to vanillin may be useful in determining the recovery of the dissolved vanillin even to an extent of any concentration between MHC and C_{max} by simple dilution with distilled water.

This is the unique advantage of the hydrotropic solubilization technique. In the concentration range of nicotinamide between 0 and 3 mol/L, three different regions were obtained using nicotinamide as a hydrotrope. It was inactive below MHC of 0.20 mol/L, above which an appreciable increase in the solubility of vanillin was found up to the C_{max} of 2.60 mol/L and beyond which there was no further solubilization effect of the hydrotrope. Therefore, nicotinamide was found to be an effective hydrotrope for vanillin in the concentration range between 0.20 and 2.60 mol/L. It was also observed that the solubilization effect of vanillin was not a linear function of the concentration of the nicotinamide solution. The solubilization effect of nicotinamide increases with increase in hydrotrope concentration and also with system temperature. A similar trend was observed in the solubilization effect of other hydrotropes, namely sodium salicylate, resorcinol and citric acid (Figs. 2-5). The highest value of solubilization enhancement factors Φs, which is the ratio of solubility values in the presence and the absence of a hydrotrope, has been observed in the case of nicotinamide as 15.07 at a system temperature of 333 K (Table-2).

TABLE-2 MAXIMUM ENHANCEMENT FACTOR FOR SOLUBILITY (Φs) OF VANILLIN				
Hydrotropes	Maximum enhancement factor for solubility (Φs)			
	T=303 K	T=313 K	T=323 K	T=333 K
Nicotinamide	9.74	10.04	10.79	15.07
Sodium salicylate	4.84	6.11	7.24	8.37
Resorcinol	3.99	4.99	5.60	6.17
Citric acid	2.87	4.21	4.98	5.66

Extraction studies: The total vanillin content in the raw material was determined separately by continuous Soxhlet extraction with ethanol. 10 g of the raw material was continuously extracted in a Soxhlet with ehanol for 12 h in order to detect the percentage of vanillin content in bean. It was estimated that *ca.* 2 % (w/w).

The vessel used for extraction studies has a height of 9 cm with a inner diameter of 7 cm fitted with a turbine impeller of diameter 1.5 cm. It has four blades and rotates at a speed of 600 rpm. A 10 g sample of vanilla bean of approximate particle size 50 μm was added to 100 mL of hydrotrope solution of known concentration in the range 0.1-3 mol/L in the glass vessel. The suspension was agitated vigorously at 600 rpm for a period of 2 h at various temperature range 303-333 K. The solution was then allowed to settle for 1 h and was subsequently filtered under vacuum within 10 min. The concentration of the dissolved vanillin was analyzed using HPLC and the interaction of vanillin with nicotinamide, sodium salicylate, resorcinol and citric acid are presented in Figs. 6-9. From the graph, it is clear that the maximum percentage (85 %) of extraction and 90 % purity was achieved in 2 h with nicotinamide at 2.6 mol/L concentration. The percentage extraction (% E) is defined as the percentage of vanillin initially present in the raw material that was extracted into a hydrotrope solution, The vanillin of highest purity was recovered from the hydrotrope solutions, probably because of the ability of the hydrotrope solutions to retain the impurities such as pigments, oleoresins and other water-soluble components.

Mass-transfer coefficient: The mass transfer coefficient of the vanillin + water system in the absence of any hydrotrope is 7.85 × 10^{-5} s^{-1} at 303 K (Table-3). The effect of different hydrotropes on the mass transfer coefficient of vanillin at different hydrotrope concentrations is also given in the same table. It can be seen that a threshold value of 0.40 mol/L is required to effect significant enhancement in the mass transfer coefficient of vanillin + water system, as observed in the case of solubility determinations. The mass transfer coefficient of

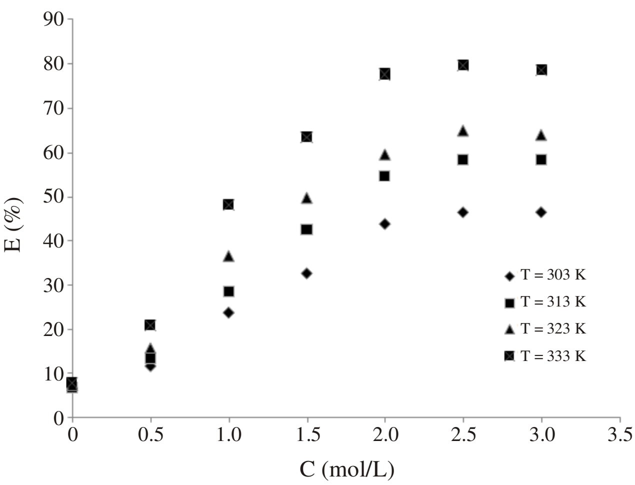

Fig. 6. Effect of nicotinamide concentration on extraction of vanillin

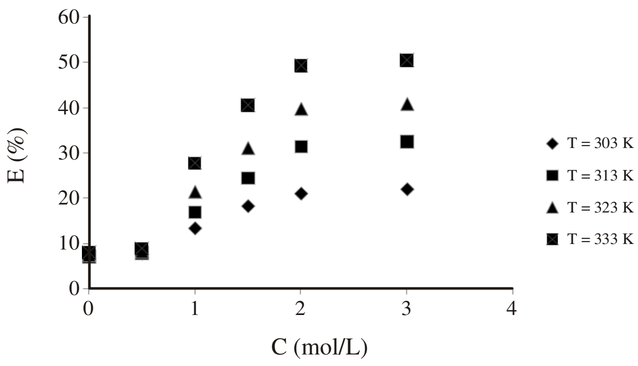

Fig. 7. Effect of citric acid concentration on extraction of vanillin

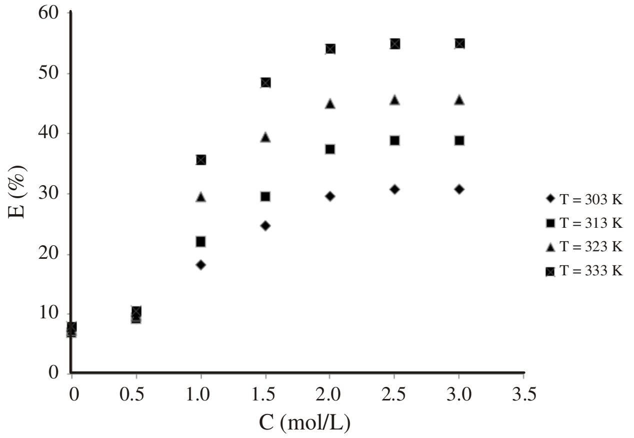

Fig. 8. Effect of resorcinal concentration on extraction of vanillin

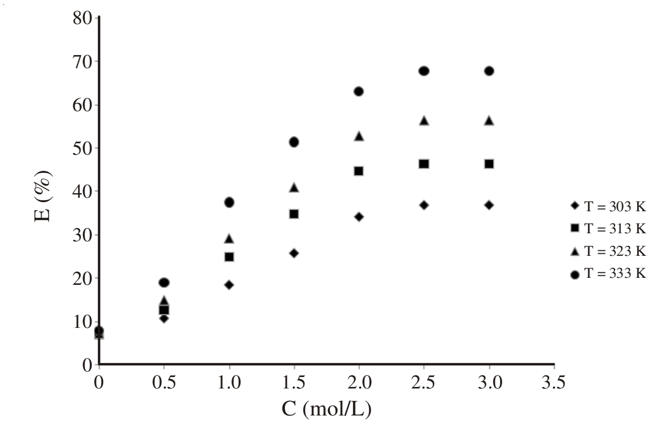

Fig. 9. Effect of sodium salicylate concentration on extraction of vanillin

vanillin + water system increases with increase in nicotinamide concentration. The maximum enhancement factor for mass transfer coefficient (Φ_{mtc}) of vanillin + water system in the presence of nicotinamide was found to be 7.71 (Table-3). A similar trend in the mass transfer coefficient enhancement of vanillin has been observed for other hydrotropes also, namely, sodium salicylate, resorcinol and citric acid.

Effectiveness of hydrotropes: The effectiveness factor of each hydrotrope with respect to vanillin at different system temperatures has been determined by analyzing the experimental solubility data for each case applying the model suggested by Setschenow[24] and later modified by Pathak and Gaikar[17] as given by the equation:

$$\log [S/S_m] = K_s [C_s - C_m] \quad (1)$$

where S and Sm are the solubility values of vanillin at maximum hydrotrope concentration C_s (same as C_{max}) and the minimum hydrotrope concentration C_m (same as MHC) respectively. The Setschenow constant Ks can be considered as a measure of the effectiveness of a hydrotrope at any given conditions of hydrotrope concentration and system temperature.

The Setschenow constant values for hydrotropes nicotinamide, sodium salicylate, resorcinol and citric acid for vanillin + water system at different system temperatures are presented in Table-4. It can be seen from the table that the highest value that has been observed was 0.50 in the case of nicotinamide as hydrotrope at 333 K.

TABLE-3
EFFECT OF HYDROTROPE CONCENTRATION (C) ON THE MASS-TRANSFER COEFFICIENT (k_La) OF VANILLIN

Hydrotropes	C (mol/L)	10^5 k_La, s^{-1}	Enhancement actor for mass-transfer coefficient, Φ_{mtc}
Nicotinamide	0	7.85 ± 0.157	-
	0.20*	8.26 ± 0.165	1.05
	0.40	12.44 ± 0.249	1.58
	1.00	22.81 ± 0.456	2.91
	1.40	39.05 ± 0.781	4.97
	1.80	48.89 ± 0.978	6.23
	2.60**	60.52 ± 1.210	7.71
Sodium salicylate	0	7.85 ± 0.157	-
	0.30*	7.98 ± 0.160	1.02
	0.50	10.22 ± 0.204	1.30
	1.00	23.71 ± 0.474	3.02
	1.40	32.24 ± 0.645	4.11
	1.80	40.05 ± 0.801	5.10
	2.40**	47.56 ± 0.951	6.05
Resorcinol	0	7.85 ± 0.157	-
	0.40*	8.10 ± 0.162	1.03
	0.60	11.26 ± 0.225	1.43
	1.00	17.55 ± 0.351	2.24
	1.40	23.91 ± 0.478	3.05
	1.80	30.66 ± 0.613	3.91
	2.20**	38.54 ± 0.771	4.91
Citric acid	0.0	7.85 ± 0.157	-
	0.50**	7.89 ± 0.158	1.01
	1.00	9.02 ± 0.180	1.14
	1.40	10.76 ± 0.215	1.37
	1.80	13.52 ± 0.270	1.72
	2.00**	17.36 ± 0.347	2.21

*MHC; **C_{max}

TABLE-4
SETSCHENOW CONSTANT [K_s] VALUES OF HYDROTROPES WITH RESPECT TO VANILLIN

Temp. (K)	Setschenow constant (K_S)			
	Nicotinamide	Sodium salicylate	Resorcinol	Citric acid
303	0.41	0.33	0.33	0.31
313	0.42	0.37	0.39	0.42
323	0.43	0.41	0.42	0.47
333	0.50	0.44	0.44	0.48

Conclusion

The solubility of vanillin which is practically insoluble in water has been increased to a maximum of 15.07 times in the presence of nicotinamide as hydrotrope at a system temperature of 333 K with corresponding increase in the mass transfer coefficient. This would be much useful in increasing the rate of output of the desired product made from vanillin. The minimum hydrotrope concentration and C_{max} values of the hydrotrope with respect to vanillin can be used for the recovery of the dissolved vanillin and hydrotrope solutions at any hydrotrope concentration between minimum hydrotrope concentration and C_{max} by simple dilution with distilled water. It was possible to extract 85 % of the material. The process was optimized with respect to concentration of hydrotrope solution and temperature required for the extraction of vanillin, this will eliminate the huge cost and energy normally involved in the separation of solubilized vanillin from its solution. Hence nicotinamide is found to be the best suitable hydrotrope for the enhancement of solubility and mass transfer coefficient of poorly soluble vanillin within the framework of the present investigation.

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