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## COMMUNICATION

# Investigation on the effect of liquid-liquid phase separation (LLPS) on nucleation and different growth stages of vanillin and bulk growth of defect free single crystals from aqueous solution - a new approach

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**In the present work, the advent of secondary liquid phase and its impact on various growth stages of vanillin single crystals in pure aqueous solution has been investigated. A new approach was implemented for the bulk growth of defect free vanillin single crystals from aqueous solution with better dimensions.**

Vanillin (4-hydroxy-3-methoxy benzaldehyde) is a savoury organic compound with wide spectrum of applications including food, liquor, pharmaceuticals, agrochemicals, non-linear optics and galvanometallic industries.<sup>1-3</sup> Crystallization of vanillin is the key unit of operation in all the above industries, which defines the crystal quality. From the previous literature, it was found that the saturated vanillin solution exhibits secondary liquid phase separation also termed as liquid-liquid phase separation (LLPS), liquid-liquid demixing or oiling out when the solute concentration and temperature exceeds their critical values.<sup>4, 5</sup> LLPS is often seen in macromolecules, and more recently, the effect of LLPS on the nucleation of small organic molecules such as  $C_{33}H_{41}Cl_2N_3O_2$  and ethyl-2-ethoxy-3-(4-hydroxyphenyl)-propanoate (EEHP) etc., have been studied.<sup>6-16</sup> Bulk growth of vanillin single crystals from solvent mixtures of chloroform-methanol, chloroform-acetone and chloroform and from melt growth process were already reported by few authors.<sup>2, 17-19</sup> So far, no report in the literature is found for the growth of bulk vanillin single crystals from pure aqueous solution and on the knowledge about metastable zone width (MSZW) of vanillin aqueous solution. Hence, a situation arises where the crystallization of vanillin in aqueous solution has to be systematically investigated in order to eliminate or suppress the impact of LLPS on the nucleation and various growth stages of bulk vanillin single crystals and hence this presents a novel approach to grow defect free bulk vanillin single crystals from pure aqueous solution by employing controlled evaporation of solvent method using eppendorf tube by which we could able to grow defect free transparent bulk vanillin single crystals through the suppression of LLPS.

Solubility of vanillin in double distilled (DD) water was determined by gravimetric method in the temperature range 15-50 °C in steps of every 2.5 °C as performed by T. Lee et al., and the curve follows the polynomial equation of  $c=0.0001t^3-0.0077t^2+0.225t-1.7017$ , where 'c' is the concentration of vanillin in g/100 mL and 't' is the saturation temperature in °C.<sup>20</sup> Polarity index (P) and pK<sub>a</sub> value of water is 9.0 and 15.7 respectively.<sup>21</sup> It is found that the solubility of vanillin increases with temperature in the range from 15 °C to 50 °C. When the temperature of the solution increased above 50 °C, the solubility of vanillin also found increased very rapidly and the solution became turbid due to the impulsive emergence of secondary liquid phase. Micro level LLPS was also found at 30 °C but it was not visible through the naked eye. Further it is noted that, the rate of LLPS increased with temperature and it is more instantaneous and visible at 50 °C. Owing to the presence of LLPS, the measurement of solubility is difficult above 50 °C.

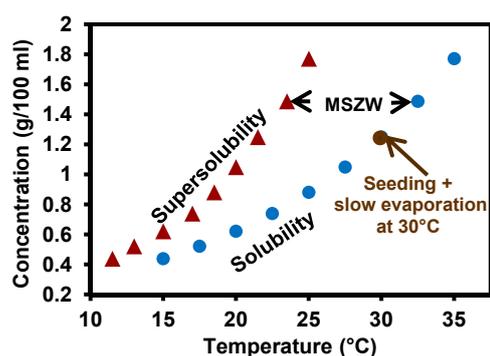
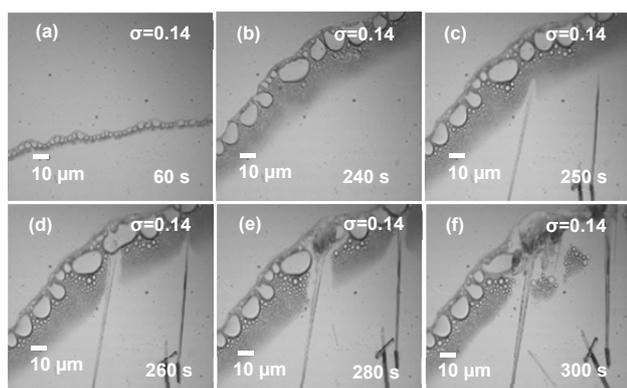


Fig. 1 Metastable zone width of vanillin aqueous solution in the temperature range 15-35 °C.

MSZW of vanillin aqueous solution at different temperature ranges 15-35 °C was determined by polythermal method and it is depicted in (Fig. 1). It is obvious from (Fig. 1a) that the rate of LLPS

increased with temperature in the range 25–35 °C and consequently it delays the nucleation of vanillin crystals to certain extent. As a result, the MSZW was found to be wider in this temperature range. At lower temperature range i.e. 15–25 °C, the rate of LLPS was approximately nil and hence the MSZW become narrowed down as shown in (Fig. 1). Hence it is worth to note that the lower temperature region is favourable for nucleation and growth of vanillin crystals with high quality.

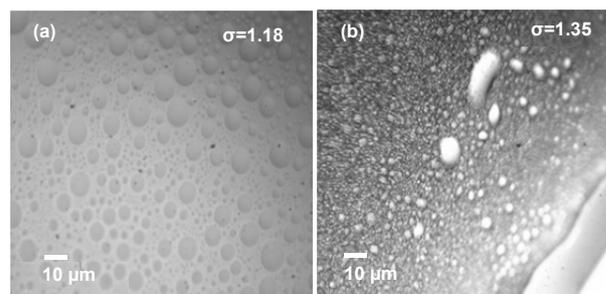
Concentration fluctuation acts as the main driving force for the occurrence of LLPS prior to crystallization during slow evaporation process at 30 °C. In aqueous solution, vanillin molecules are getting self-associated through the stacking interactions which may also lead to LLPS.<sup>22</sup> In case of slow evaporation, the supersaturation generated within the mother liquor was low and it was not suffice to produce nucleation of vanillin crystals. Moreover the rate of LLPS was found to be increased with aging of the solution. Because of this aging, the solution produces gravity driven LLPS. Hence by this traditional approach (i.e. slow evaporation), the self-nucleation of vanillin crystals from pure aqueous solution is not possible. When the saturated solution was allowed for fast evaporation at 30 °C, the rate of LLPS was increased with time. The existence of LLPS and its influence on the different growth stages of vanillin crystals in aqueous solution is presented in (Fig. 2).



**Fig. 2** The effect of LLPS on the growing crystal (a) advent of LLPS (b) coalescence of oil droplets (c) crystallization in continuous solute-lean liquid phase (d) contact of crystals at the interface of two liquid phases (e) crystallization of oil droplets and (f) transparency loss in growing crystal.

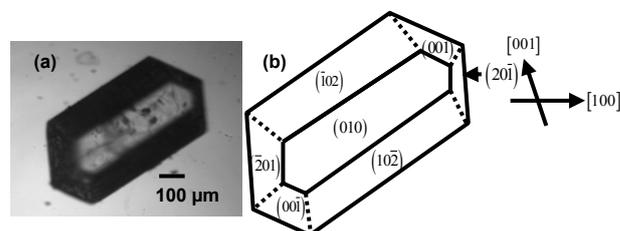
When the supersaturation was  $\sigma=0.14$ , discontinuous micro droplets appeared within the mother liquor. The droplets and the continuous liquid phase differ in solute concentration.<sup>5</sup> The droplets were small, highly viscous, rich in solute and less in number as shown in (Fig. 2a) at the time interval of 60 s. With increase in time from 0 s to 240 s, they increase in number, coalesce with each other and grow larger in size as shown in (Fig. 2b). According to the ‘‘Ostwald’s rule of Stages’’, the secondary liquid phase is metastable, there was no nucleation found inside the droplets.<sup>23</sup> The droplets always required stimulation from an external force like seeding. Eventually, nucleation of vanillin had occurred inside the solute-lean, low viscous continuous liquid phase as shown in (Fig. 2c) at 250 s and the nucleated crystals grew at a slower rate and were optically

transparent. When the growing crystals come in contact with the interface of solute-rich oil droplets at 260 s, rapid crystallization occurs inside the droplets as shown in (Fig. 2d) and faster growth is observed. The crystals grew by consuming the solute from droplets and they lose their transparency within 20 s as shown in (Fig. 2e) and (Fig. 2f). The influence of LLPS on nucleation is very low when the supersaturation is inferior. At higher supersaturation  $\sigma=1.18$  and  $\sigma=1.35$ , the rate of LLPS increases with increase in supersaturation and it delays the nucleation up to a greater extent with respect to the supersaturation. The LLPS observed at supersaturation  $\sigma=1.18$  and  $\sigma=1.35$  are shown in (Fig. 3a) and (Fig. 3b) respectively.



**Fig. 3** (a) LLPS at supersaturation  $\sigma=1.18$  and (b) LLPS at supersaturation  $\sigma=1.35$

Slow cooling method was adopted to induce the homogeneous nucleation of vanillin crystals. By continuous reduction of temperature from 40 to 30 °C at the rate of 2 °C/h, the solution gets necessary supersaturation and the crystals were nucleated and grew up to the equilibrium concentration.

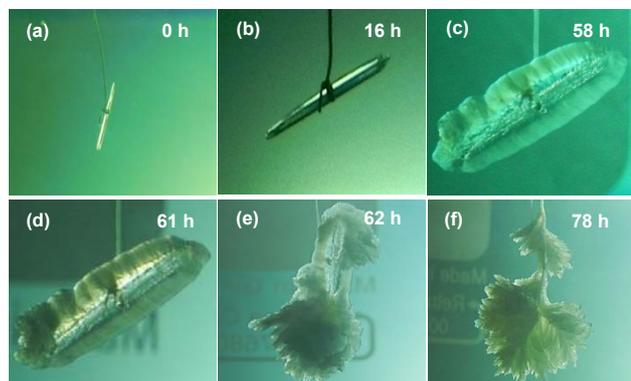


**Fig. 4** (a) Microscopic image of vanillin single crystal grown by slow cooling and (b) its schematic morphology.

One such vanillin single crystal grown by slow cooling with its habitual morphology and its schematic are presented in (Fig. 4a) and (Fig. 4b) respectively. The grown transparent crystals were used as the seeds to grow bulk vanillin single crystals in further experiments. The faces  $(\bar{2}01)$  and  $(001)$  have grown at a faster rate compared to the face  $(\bar{1}02)$  whose growth is faster than that of the face  $(010)$ . The face  $(010)$  is the only face that grows at a slower rate and is found to exist as the prominent face in the fully grown crystal. The morphological importance of the prominent growth faces are in the order  $(010) > (\bar{1}02) > (001)$ .

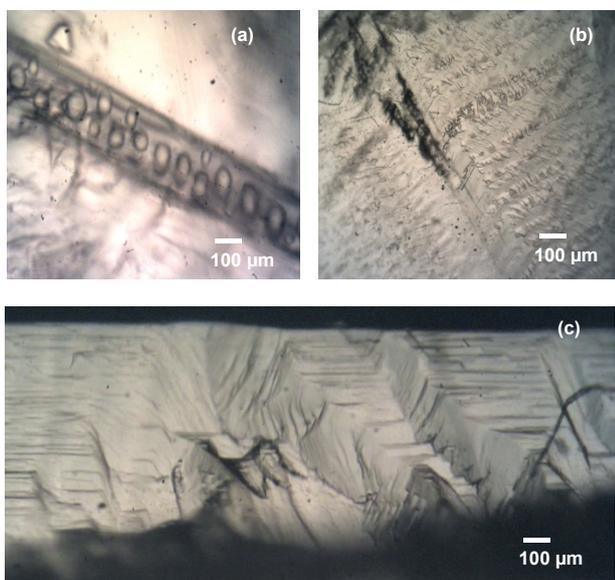
Bulk single crystals of vanillin were grown by slow cooling method using two different techniques: (i) without seed rotation and (ii) with seed rotation. In both the experiments, the aqueous solutions of vanillin were saturated at 42.5 °C based on the solubility data. Seed crystal used in the growth experiment by the absence of

seed rotation is shown in (Fig. 5a). From our experiment it can be seen that the growth of vanillin crystal had occurred in four different stages. When the temperature of the solution was reduced at a rate of  $0.01\text{ }^{\circ}\text{C}/\text{h}$  from  $42.50$  to  $42.34\text{ }^{\circ}\text{C}$ , seed crystal grew at a faster rate with defined morphology as shown in photograph (Fig. 5b).



**Fig. 5** Growth progression of vanillin single crystal in water without seed rotation by slow cooling (a) seed crystal (b) growth of the seed with transparency and perfect morphology (c) re-dissolution of the edges and oil entrapment towards the center of the growing crystal (d) terrace formation (e) surface nucleation of leafy spike-like dendrites at the bottom and top, and (f) grown leafy spike-like dendrites at the end of the experiment.

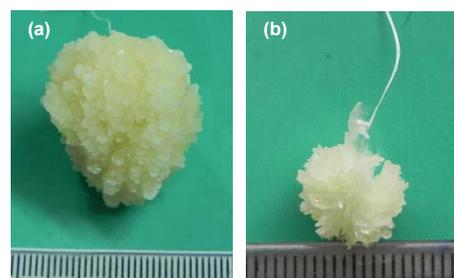
In this first stage i.e., from the beginning of the experiment (0 h) to 16 h, fast growth was found along  $[001]$  direction and slow growth was observed along  $[010]$  direction.



**Fig. 6** (a) Inclusion of oil inside the seed crystal (b) growth layers of vanillin crystal and (c) terrace formation along the edges of the crystal.

In second stage i.e. 16 h-57 h, growth rate of the crystal was found to be very low while reducing the temperature of the solution from  $42.34$  to  $39.29\text{ }^{\circ}\text{C}$  at a rate of  $0.02\text{-}0.1\text{ }^{\circ}\text{C}/\text{h}$ . In this stage, growth of the crystal was terminated even at higher cooling rate (i.e. generation of higher supersaturation within the system). Growth inhibition is due

to the enrichment of LLPS and the requirement of relatively higher supersaturation for the crystallization of oil droplets. In third stage i.e. 57-63 h, on further reducing the temperature, sharp crystal edges were slightly re-dissolved and end up with curved edges which also lost its transparency as shown in (Fig. 5c). Dissolution of the crystal edges is due to the inclusion of solute-rich dense oil droplets on the preferred orientation of growth units observed in the experiment is shown in (Fig. 6a). Growth layers and terraces formed during growth are shown in (Fig. 6b) and (Fig. 6c) respectively. Facets of habit faces and terraced regions formed in the intermediate were grown until a single edge between the two habit faces involving the re-dissolution, was formed according to the theorem of Herring.<sup>24</sup> In our present work, no single edge between the two faces was formed till the end of the experiment, as a result, the terraced surfaces continues to grow. At first, the deformation of the crystal structure was slowly penetrated towards the center of the growing crystal as in (Fig. 5d) and later it is emerging out from the center as in (Fig. 5e). The rate of deformation was found to be very fast and it ends up within 4 h (i.e. from 58 to 62 h). In fourth stage i.e. 63-78 h, while further reducing the temperature, growth on terraced surfaces favours the entrapment of oil droplets and leads to their crystallization on the surface of the parent crystal following the mechanism of contact nucleation by surface breeding.<sup>25</sup> The aggregated mass deposition produced leafy spike-like dendrites at the end as in photograph (Fig. 5f). The end product lost its optical transparency and turned into pale yellow colour.

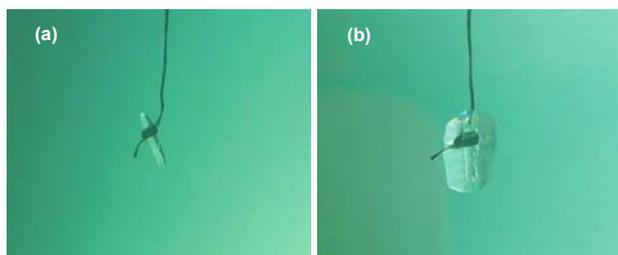


**Fig. 7** Grown single crystals of vanillin with leafy spike-like dendrites (a) crystal grown by slow cooling with seed rotation and (b) crystal grown by slow cooling without seed rotation.

All the above four stages of growth were also observed in the case of bulk growth from aqueous solution with seed rotation at the rate of 30 rpm. Photographs of the vanillin single crystals harvested from aqueous solution with and without seed rotation are shown in (Fig. 7a) and (Fig. 7b) respectively. Even though the same cooling rate was employed, growth of the crystal was ceased at  $39.96\text{ }^{\circ}\text{C}$  which is so earlier compared to that of the crystal grown without seed rotation. Mass of the seed crystal (i.e.  $0.008\text{ g}$ ) used in both the above experiments was same but the mass of the harvested crystal grown with seed rotation was  $7.1\text{ g}$  and for the crystal grown without seed rotation was  $2.4\text{ g}$ . Large discrepancy in these results clearly indicates that, seed rotation influences the movement of oil droplets and enhances their crystallization on the growing crystal with larger extend. It was also observed that, the impact of LLPS was greater on

the growth faces of bulk crystal in aqueous solution saturated at 42.5 °C.

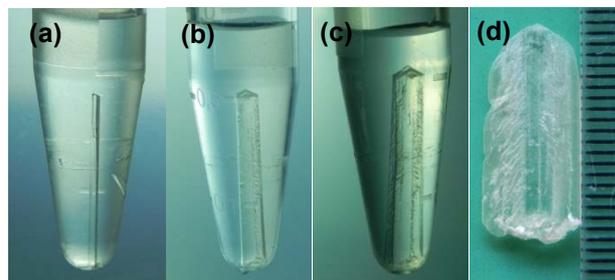
In order to study the effect of LLPS at little lower temperature, one more experiment was performed with the aqueous solution saturated at 40 °C. The growth experiment was performed in the absence of seed rotation. (Fig. 8a) shows the morphology of the seed crystal used in this experiment. The same cooling rate (i.e. 0.01 °C/h) was followed as used in the previous experiments. Slow and continuous growth was observed until the solution reaches 35 °C. Further, cooling rate was increased from 0.02 to 0.2 °C, there was no growth observed till the end of this experiment. The grown crystal has good quality and small in size as shown in (Fig. 8b). From this experiment, it has been confirmed that the saturation temperature play a significant role in the bulk growth of vanillin crystals in aqueous solution.



**Fig. 8** (a) Seed crystal used in the bulk growth of vanillin single crystal from the aqueous solution saturated at 40 °C by slow cooling in the absence of seed rotation and (b) grown single crystal.

In an alternative effort to increase the growth rate and to suppress the impact of LLPS, a novel method was adopted, in which the seed crystal with dimension (1.6 cm x 0.1 cm x 0.1 cm) as shown in (Fig. 9a) was kept inside the eppendorph tube (1.5 mL capacity) containing the aqueous solution of vanillin and the experimental condition of seeding is indicated in (Fig.1). The solution was saturated at 30 °C, kept at equilibrium for 6 h and filtered using an on-line filter with porosity 15-25 µm. The eppendorph tube was kept inside a dust free evaporation chamber maintained at 30 °C. The solution was changed for every 5 days as its level lowered due to evaporation. The dimensions of the crystals were measured and examined under optical microscope for the changes in its growth at every time whenever the solution was changed. As the growth of the crystal was commendable and it could not be further grown inside the eppendorph tube due to its dimensional incapability to sufficiently hold further growth of the crystal towards the bottom. So the crystal was shifted to another round bottom cuvette with a larger diameter which could provide sufficient space for the crystal to occupy during its growth. The same procedure of renewing the solution was followed regularly. The evaporation takes place only through a small orifice made on the lid of the eppendorph tube and the continuous renewal of solution at regular intervals leads to lower and uniform generation of supersaturation ( $\sigma=0.0015$ ) in the mother liquor. Due to this continuous generation of lower supersaturation, growth rate of the crystal was found to be constant throughout the experiment and the impact of LLPS on the growth faces was less frequent. Faster growth was observed along the [001] direction and

slower growth was observed along the [010] direction. The growth progression of vanillin single crystal is shown in (Fig. 9). The crystal grown with sharp edges was found to be transparent; free from crystal deformation with larger dimension of about (2.1 cm x 0.9 cm x 0.1 cm).



**Fig. 9** Various growth steps of vanillin single crystal in eppendorph tube (a) Seed crystal (b) after 21 days and (c) after 35 days (d) after harvested.

The problems encountered in the previous experiments were successfully resolved by adopting this novel approach. Moreover, we could able to grow bulk vanillin single crystal with very small volume of the aqueous solution when compared to previous cases.

To examine the crystalline purity, thermal stability and chemical purity, the grown crystals were subjected to powder X-ray diffraction (PXRD), differential scanning calorimetry (DSC) and Fourier transform infra-red spectroscopy (FTIR) analysis respectively. From PXRD analysis, it was confirmed that the crystal with leafy spike-like morphology possess highly ordered crystalline phase without any inclusion of foreign particles in its crystal lattice. DSC analysis shows that there was no significant variation in the melting point of the crystals grown from different methods and no phase transition was observed. Crystals were thermally quite stable up to their melting point FTIR analysis infers that all the grown single crystals of vanillin have the same type of chemical entity, chemically pure and there was no possibility for the adherence of different chemical species on the growth surfaces of the crystal. Characterization results reveal the grown crystals to be the Form I polymorph of vanillin.<sup>3, 17, 26</sup>

## Conclusions

LLPS occurred in the saturated aqueous solution of vanillin greatly affects the nucleation, growth and optical quality of the crystals. Temperature, concentration, stirring and aging of the solution plays a key role in controlling the occurrence of LLPS. MSZW of vanillin aqueous solution and the bulk growth of defect free vanillin single crystal in aqueous solution have been reported for the first time. The crystals grown by slow cooling method involve four different growth stages and they lost their transparency due to the inclusion of oil droplets on the preferred orientation of growth units. The traditional approaches, slow evaporation and slow cooling (saturation at higher temperature) are not fruitful in suppressing the augmentation of LLPS and in the growth of transparent single crystals. A new method proposed in this work is the promising one to eliminate the effect of oil on the growth surfaces of vanillin crystals in aqueous solution. So far, the bulk single crystal of vanillin with dimension of about 2.1 cm x

0.9 cm x 0.1 cm was not grown by any of the techniques and methods reported earlier in the existing literature. Additional advantages of this new method are it is easy to perform at room temperature, requires only small volume of the solution and very economy no need for sophisticated instruments. The characterization results of the grown crystals indicate that the deformed mass of aggregation, transparent crystals grown by slow cooling and controlled evaporation are pure in their crystalline and chemical nature. The leafy spike-like dendrites are formed only by the trajectory, during growth of the facets and terraces not by the inclusion of foreign impurities. These results also indicate that there is no undesirable chemical reaction (i.e. contamination during processing) takes place between the solute and solvent during crystallization of vanillin crystals from aqueous solution. Seeding at lower supersaturation and lower temperature with lesser volume of the solution by this newer approach we could able to grow defect free bulk vanillin single crystal from aqueous solution.

## Notes and references

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