

# Partial molar volume reduction of solvent for solute crystallization using carbon dioxide as antisolvent

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## Abstract

The gas antisolvent crystallization (GASC) process using dense carbon dioxide ( $\text{CO}_2$ ) as antisolvent is particularly useful for purification and micronization of thermo-labile bioactive solid substances. Conventionally, the GASC process is characterized by the relative total volume expansion or the relative molar volume expansion of the solution. A new criterion is proposed in this work in terms of the relative partial molar volume reduction (RPMVR) of the solvent for selection of the solvent and the optimum process condition for the GASC process, as it directly gives a measure of the fraction of the dissolved solute crystallized. The solute solubility is proportional to the partial molar volume of the solvent,  $\bar{v}_2$  which drastically decreases at a high  $\text{CO}_2$  dissolution. This is attributed to clustering of  $\text{CO}_2$  molecules around the solvent molecules causing the loss of solvent power. This results in the desired antisolvent effect for lowering the solute solubility.  $\bar{v}_2$  has been calculated for a large number of solvent– $\text{CO}_2$  liquid mixtures using the Peng–Robinson equation of state. It has been observed that  $\bar{v}_2$  drastically reduces at a high value of  $x_1$ , irrespective of the fact whether the solvent density is higher or lower than that of the  $\text{CO}_2$ . The solute solubility has been predicted from its value at the ambient pressure and the ratio of the partial molar volumes of the solvent with and without  $\text{CO}_2$  dissolved in it. The predicted solubility of  $\beta$ -carotene in ethyl acetate with variation of  $x_1$  at 298 K has been found to compare well with the experimentally observed trend of the GASC process.

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**Keywords:** Gas antisolvent crystallization (GASC); Partial molar volume of solvent; Relative partial molar volume reduction (RPMVR); Solute solubility;  $\text{CO}_2$  Dissolution; Antisolvent effect

## 1. Introduction

In recent years, the gas antisolvent crystallization (GASC) has been found to be very effective for purification and micronization of thermolabile and bioactive solid pharmaceuticals. This process

involves dissolution of the solid substrate in an organic solvent and subsequently dissolving dense carbon dioxide in the solution for reduction of its solvent power in order to precipitate out the dissolved solute in the form of crystals. Carbon dioxide is the most advantageous antisolvent, as it is inert, nontoxic and generally regarded as safe (GRAS). It is a gas at ambient condition and its recovery is very easy. As  $\text{CO}_2$  has very high

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**Nomenclature**

$k_{12}$	binary interaction constant between CO <sub>2</sub> (1) and solvent (2)
$P$	pressure
$T$	temperature
$V$	total volume of liquid solution
$v$	molar volume of solution
$v_1$	molar volume of component 1
$v_2$	molar volume of component 2
$\bar{v}_1$	partial molar volume of component 1 in a binary mixture of 1 and 2
$\bar{v}_2$	partial molar volume of component 2 in a binary mixture of 1 and 2
$x_1$	mole fraction of component, 1 in liquid phase on solute-free basis
$x_2$	mole fraction of component, 2 in liquid phase on solute-free basis
$x_3$	mole fraction of component, 3 in liquid phase on antisolvent-free basis
1, 2, 3	antisolvent, solvent, solute components

solubility in organic solvents, its dissolution in the solution causes significant reduction in its partial molar volume and cohesive energy density, lowering its solvent power for the solid solute and thus causes its crystallization at near-ambient temperature.

In the case of purification by GASC, the desired solid component is selectively crystallized out, leaving behind the remaining components in the solution. For micronization by using GASC, the dissolved pure solute is completely recrystallized to produce micro/nano particles having the desired morphology, size and size distribution. There are two ways of contacting the solution with carbon dioxide, namely: (i) bubbling carbon dioxide to solution in a batch mode; and (ii) spraying the solution into a fast flowing stream of dense carbon dioxide in a continuous mode. The rapid dissolution of CO<sub>2</sub> in the solution causes sequential precipitation of the dissolved solutes depending on the nature of solid–solvent interaction, in the first method. On the other hand, the two-way diffusion between CO<sub>2</sub> and the ultra-fine droplet of dilute solution in the latter method causes rapid crystallization of the solute in the droplet first, followed by complete evaporation of the solvent and results in the formation of ultra-fine particles.

The present article introduces a new thermodynamic concept for selection of solvent and process conditions for GASC in terms of the antisolvent-induced partial molar volume reduc-

tion of the solvent and presents a method for prediction of the saturated solute solubility for crystallization in terms of the CO<sub>2</sub> dissolution in the solution and temperature, as the controlling parameters.

## 2. Thermodynamic analysis

It is necessary to find the appropriate solvent for the given solute and the optimum temperature and pressure conditions for selective crystallization of the solute from its solution, at which the antisolvent is either totally miscible or has a very high solubility in the solvent. The antisolvent and solvent are completely miscible as the binary mixture reaches the critical point. There is an exponential increase in the total volume of the solution with increasing dissolution of the antisolvent. Originally, the relative total volume expansion of the solvent was considered as the criterion for crystallization and was characterized [1] as:

$$\frac{\Delta V}{V} = \frac{V_L(T, P, x_1) - V_2(T, P_0)}{V_2(T, P_0)} \quad (1)$$

where  $V_L$  is the total volume of the liquid phase,  $V_2$  is the total volume of the pure solvent at the same temperature and reference pressure  $P_0$  (normally, atmospheric pressure). This can be rearranged [2] as:

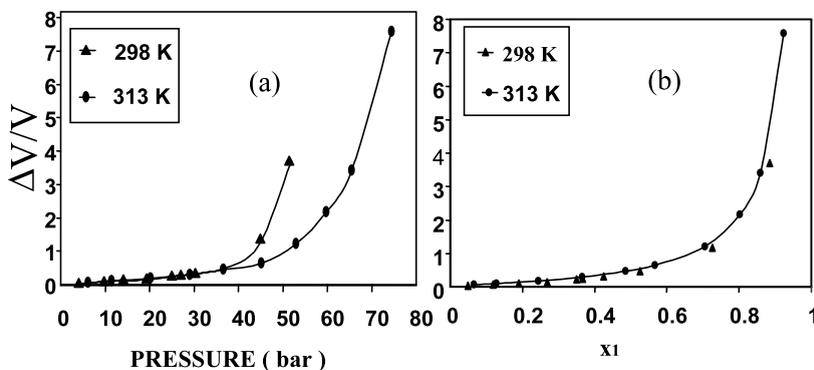


Fig. 1. (a) Effect of pressure on total volume expansion of hexane. (b) Effect of  $\text{CO}_2$  dissolution on total volume expansion of hexane.

$$\frac{\Delta V}{V} = \frac{1}{1 - x_1} \cdot \frac{v_L(T, P, x_1)}{v_2(T, P_0)} - 1 \quad (2)$$

where  $v_L$  is the molar volume of the liquid solution and  $v_2$  is the molar volume of the solvent, 2.  $x_1$  denotes the mole fraction of antisolvent, 1 in the solution. As shown in Fig. 1(a) the isothermal plots of  $(\Delta V/V)$  versus  $P$  are different for different temperatures. They are also different for different solvents though the behavior is the same [1].  $(\Delta V/V)$  increases with pressure slowly at low pressures and then rapidly increases at high pressures. With the appearance of liquid–liquid immiscibility, the volume expansion remains invariant with increasing pressure. However, the isothermal plots of  $(\Delta V/V)$  versus  $x_1$  coincide for all solvents, as  $v_L/v_2$  is close to unity for all solvents. In other words, the relative total volume expansion is unable to recognize the difference between the solvents for a given antisolvent. Similarly, the relative total volume expansion versus  $x_1$  coincides for different temperatures, as shown in Fig. 1(b). As this parameter cannot distinguish the difference between the solvents and temperatures with a variation in  $x_1$ , the key controlling parameter, it cannot be considered appropriate for characterization of the GASC process.

Subsequently, the relative molar volume expansion was considered [2] to be the more appropriate parameter and a minimum in the plot of the relative molar volume expansion versus pressure or  $x_1$ , was suggested as the criterion for ascertaining the optimum condition for the GASC process. The relative molar volume expansion was defined

as:

$$\frac{\Delta v}{v} = \frac{v_L(T, P, x_1)}{v_2(T, P_0)} - 1 \quad (3)$$

Its value is negative as the molar volume decreases with  $\text{CO}_2$  dissolution. The behavior of the relative molar volume expansion for hexane– $\text{CO}_2$  system at two temperatures is shown in Fig. 2. It may be noted that there is no expansion as such, as the molar volume of the solution  $v$  decreases with  $x_1$ . In general, the molar volume of the solution is mostly less than that of the pure solvent  $v_2$ , except for a very high value of  $x_1$  in organic solvents irrespective of the fact whether  $v_2 > v_1$  or  $v_2 < v_1$ .

Further, with increasing  $\text{CO}_2$  dissolution, the liquid molar volume of solution,  $v$  decreases as  $\bar{v}_1 < \bar{v}_2$ . It passes through a minimum, when  $\bar{v}_1 = \bar{v}_2$  and then increases if  $\bar{v}_1 > \bar{v}_2$  as:

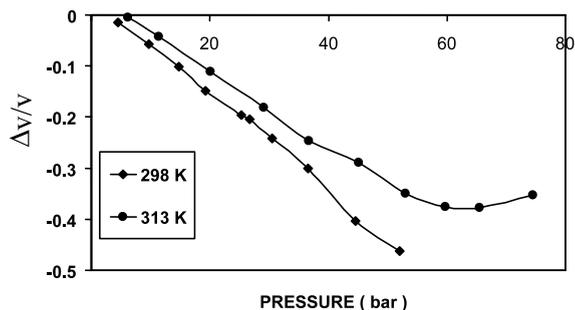


Fig. 2. Effect of pressure on molar volume of expansion of hexane.

$$\left(\frac{\partial v}{\partial x_1}\right)_{P,T} = (\bar{v}_1 - \bar{v}_2)$$

$$(4) \quad \frac{dv}{dx_1} = \left(\frac{\partial v}{\partial P}\right)_{x_1,T} \frac{dP}{dx_1} + \left(\frac{\partial v}{\partial x_1}\right)_{P,T} \quad (5)$$

Also

As can be seen from the R.H.S. of Eq. (5), the first

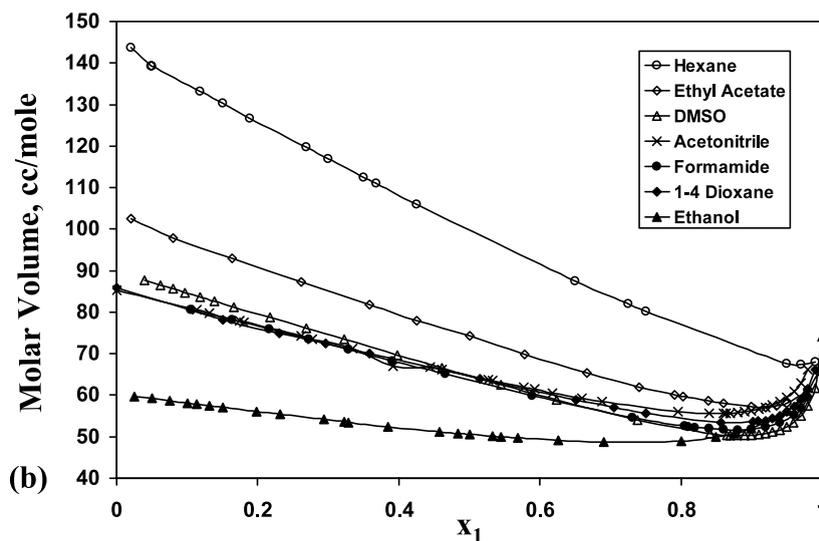
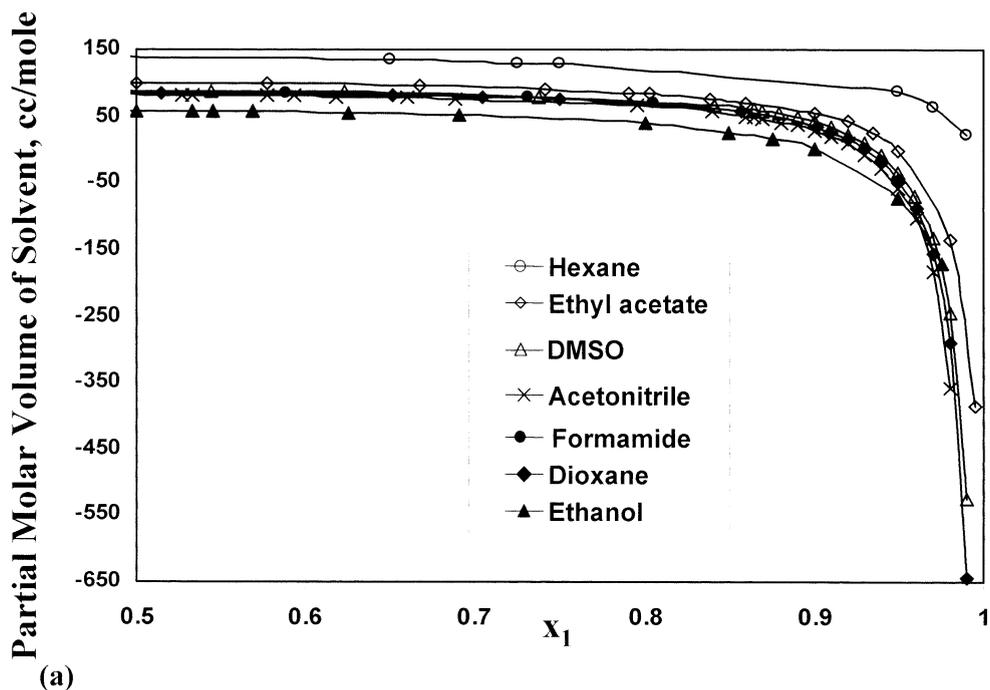


Fig. 3. (a) Partial molar volumes of different solvents at 298 K with CO<sub>2</sub>. (b) Molar volumes of different solvent–CO<sub>2</sub> systems at 298 K.

Table 1  
Interaction constants in the P–R equation [1]

System	$K_{ij}$	$I_{ij}$
CO <sub>2</sub> +Acetonitrile	0.07	0
CO <sub>2</sub> +1,4 Dioxane	-0.05	-0.05
CO <sub>2</sub> +Ethyl acetate	-0.02	0.01
CO <sub>2</sub> +Dimethyl sulfoxide	0.015	-0.025
CO <sub>2</sub> + <i>N,N</i> -Dimethyl formamide	0.017	-0.043
CO <sub>2</sub> +Ethanol	0.089	0

term is negative and small and the second term is initially negative and later positive. Accordingly, the liquid molar volume initially decreases and later increases with CO<sub>2</sub> dissolution due to the increase of the second term at high pressures, close to the vapor pressure of the antisolvent at subcritical temperature or near the mixture critical pressure of the solvent–CO<sub>2</sub> system at supercritical temperatures. The total volume expansion behavior also shows an exponential increase with pressure at such pressures. It will be seen later that the occurrence of nucleation at such high pressures is essentially observed due to the fact that the partial molar volume of the solvent sharply diminishes with the high CO<sub>2</sub> dissolution at such pressures, as a result of clustering of CO<sub>2</sub> mole-

cules around the solvent molecule, rather than due to the total volume expansion per se.

It may be noted that, even in the case of the systems comprising denser solvent and lighter antisolvent combinations (e.g. ethanol–CO<sub>2</sub> system), i.e. for  $v_1 > v_2$ , the liquid molar volume of solution  $v$  may first marginally decrease with  $x_1$  and then exceed  $v_2$  at a high value of  $x_1$  and the occurrence of the minimum value (where  $v = \bar{v}_1 = \bar{v}_2$ ) of the molar volume may not be prominent at all.

For other organic liquid solvents, e.g. hexane (where  $v_1 < v_2$ ), the minimum value of the molar volume may not be prominent, as it flattens out over a wide range of  $x_1$ . As a result, the relative molar volume expansion may remain steady or increase slowly at a very high value of CO<sub>2</sub> dissolution. In view of such possibilities, the general criterion of selecting optimum process condition at the minimum value of the molar volume, as suggested by Badilla et al. [2] is inappropriate. Actually, the nucleation of the solute may occur at a value of CO<sub>2</sub> dissolution and temperature irrespective of the condition at which the molar volume is minimum. Therefore, both the relative total volume expansion and relative molar volume expansion are considered

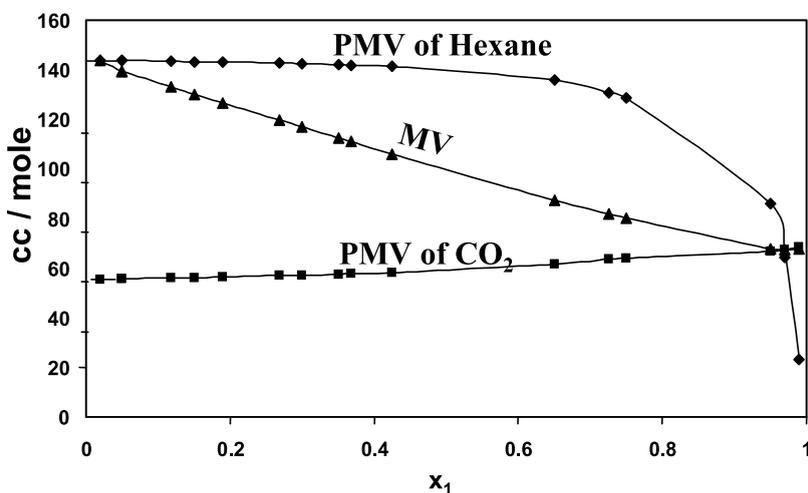


Fig. 4. Antisolvent effect on molar volume and partial molar volumes for hexane–CO<sub>2</sub> system at 298 K.

as inappropriate criteria for characterizing the GASC process.

### 3. Proposed criterion for GASC

The CO<sub>2</sub> solubility at which  $\bar{v}_2$  is sharply decreased corresponds to the drastic reduction of solute solubility and the optimum condition for GASC. Hence, a new criterion is proposed in terms of the partial molar volume reduction of the solvent, in order to select the conditions of temperature and pressure and the key parameter for selective crystallization by antisolvent dissolution is the relative partial molar volume reduction (RPMVR), which is defined as:

$$-\frac{\Delta\bar{v}}{v} = 1 - \frac{\bar{v}_2}{v_2} \quad (6)$$

The decrease in solute solubility with a reduction in the partial molar volume of the solvent is given by the relation of the equilibrium solubility of a solid solute in a liquid solvent (on antisolvent-free basis) which may be calculated as:

$$x_3(T, P) = \frac{\bar{v}_2(T, P, x_1)}{\bar{v}_2(T, 1, O)} x_3(T, 1) \quad (7)$$

where  $x_3$  and  $x_1$  are the mole fractions of the solute (on antisolvent-free basis) and the antisolvent (on solute-free basis), respectively and  $\bar{v}_2$  is the partial molar volume of the solvent in the binary (solvent–antisolvent) mixture [3]. Both solute–

solvent and solvent–antisolvent interactions are considered in the terms,  $x_3$  and  $\bar{v}_2$ , respectively.  $\bar{v}_2$  is calculated from the following relation assuming that there is negligible influence of the presence of solute in the solution:

$$\begin{aligned} \bar{v}_2 &= \left[ v - x_1 \left( \frac{\partial v}{\partial x_1} \right)_{P,T} \right] \\ &= \left[ v + (1 - x_2) \left( \frac{\partial v}{\partial x_2} \right)_{P,T} \right] \end{aligned} \quad (8)$$

where  $v$  is the liquid molar volume calculated from the Peng–Robinson equation of state with interaction constant,  $k_{12}$  regressed from the vapor–liquid equilibrium data [4].

Another parameter, the relative molar volume reduction (RMVR) has been defined in this work as the negative of the relative molar volume of expansion (Eq. (3)) for the sake of comparison with the proposed new criterion, RPMVR (Eq. (6)). The advantage of this parameter, RMVR, is that it is always positive as in the case of RPMVR.

## 4. Results and discussion

### 4.1. Partial molar volume

In the present work,  $k_{12}$  for CO<sub>2</sub>–hexane system [5] has been regressed to be 0.112 and 0.125 at 298 and 313 K, respectively.  $k_{12}$  and V–L–E data for other binary systems (listed in Table 1) have been taken from the literature [1].

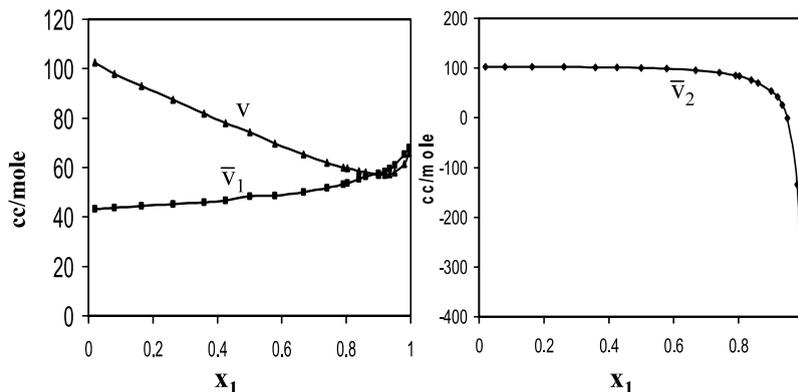


Fig. 5. Antisolvent effect on molar volume and partial molar volumes at 298 K for ethyl acetate–CO<sub>2</sub> system.

$\bar{v}_2$  slowly decreases with increasing CO<sub>2</sub> mole fraction and drastically reduces at high values of CO<sub>2</sub> mole fraction, as can be seen from Fig. 3(a) for a large number of solvents at 298 K, whereas the variation in the molar volume of the solution at a high CO<sub>2</sub> dissolution is relatively less sensitive, as can be seen from Fig. 3(b). The molar volume,  $v$  and the partial molar volumes of solvent and CO<sub>2</sub> are compared in the same plots, Figs. 4 and 5 for hexane–CO<sub>2</sub> and ethyl acetate–CO<sub>2</sub> systems,

respectively. It can be observed that the plots of  $\bar{v}_1$ ,  $v$  and  $\bar{v}_2$  intersect at a high value of  $x_1$  at which  $v$  tends to pass through a minimum, though not prominently, as all three values are equal at this value of  $x_1$ . In other words, the value of  $x_1$  corresponding to the minimum in the molar volume can only be obtained graphically from the point of intersection of these three plots.

At pressures > 50 bar, the liquid phase is mostly CO<sub>2</sub> (> 80% by weight at near ambient tempera-

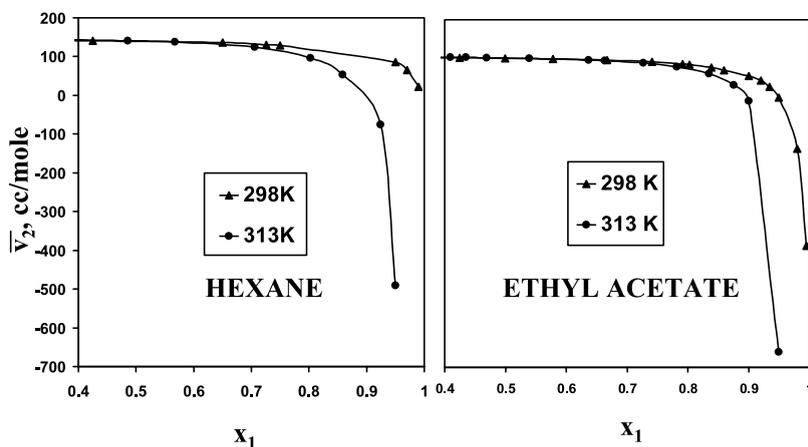


Fig. 6. Effect of temperature on PMV of hexane and ethyl acetate.

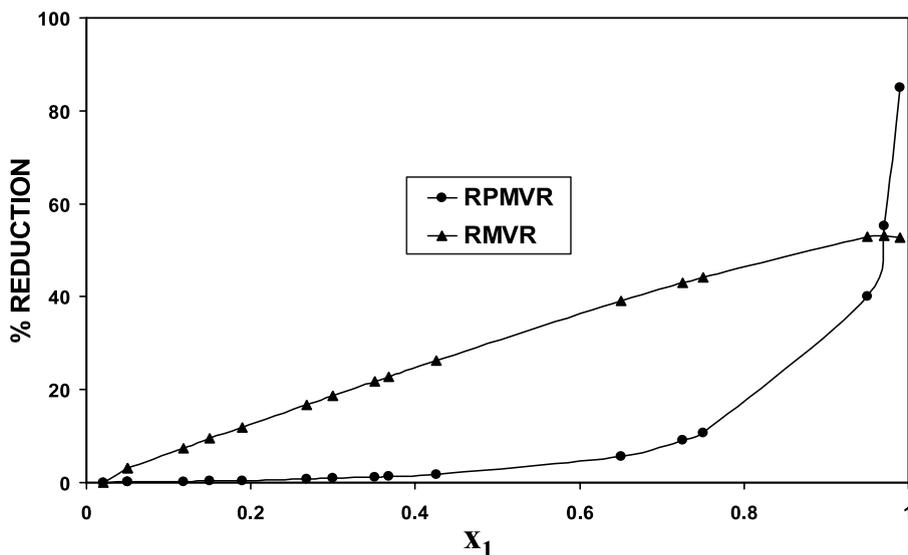


Fig. 7. Comparison of RMVR and RPMVR for hexane–CO<sub>2</sub> system at 298 K.

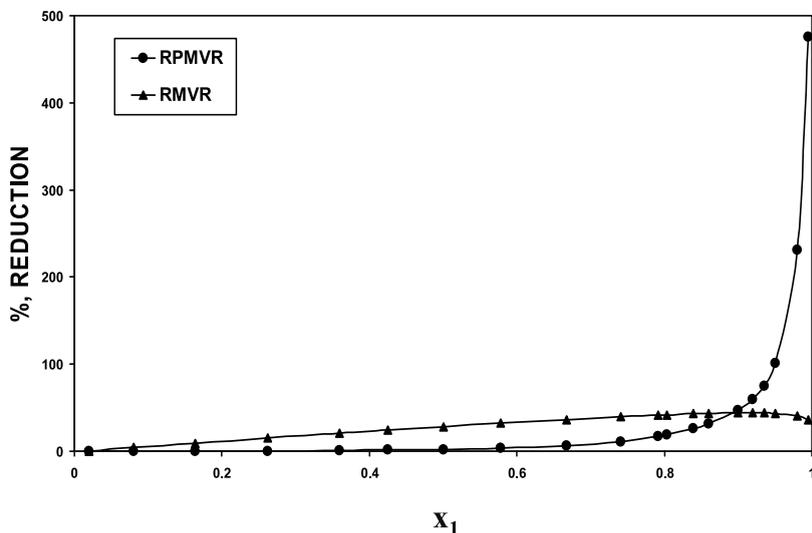


Fig. 8. Comparison of RMVR and RPMVR for ethyl acetate–CO<sub>2</sub> system at 298 K.

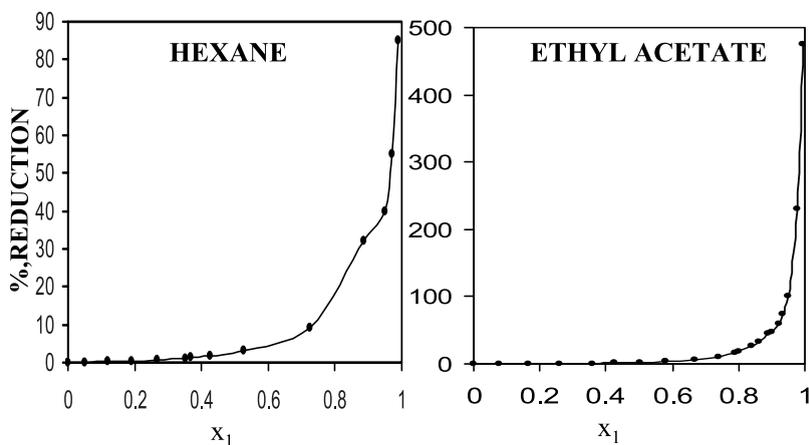


Fig. 9. Comparison of RPMVR of two solvents at 298 K.

ture) and contains very much less solvent. As a result, CO<sub>2</sub> molecules cluster around the solvent molecules causing reduction in  $\bar{v}_2$ , due to the effect of pressure on CO<sub>2</sub> volume. This clustering leads to the dislocation of the solute–solvent bonds and thus reduces the solvent’s strength to retain the solute in solution, allowing coalescence of the secluded solute molecules. Thus, the partial molar volume reduction of the solvent causes the decrease in the solute solubility and the solvent–

antisolvent interaction is more important than the solute–antisolvent interaction.

However,  $\bar{v}_1$  slightly increases initially and later steeply, as the liquid phase is enriched with CO<sub>2</sub>. With increasing CO<sub>2</sub> dissolution, the liquid molar volume initially decreases for  $\bar{v}_1 < \bar{v}_2$ , passes through a minimum for  $v = \bar{v}_1 = \bar{v}_2$  and then increases when  $\bar{v}_1 > \bar{v}_2$ , as  $v = x_1 \bar{v}_1 + x_2 \bar{v}_2$ .

Similarly, the partial molar volume of the solvent,  $\bar{v}_2$  decreases with an increase in tempera-

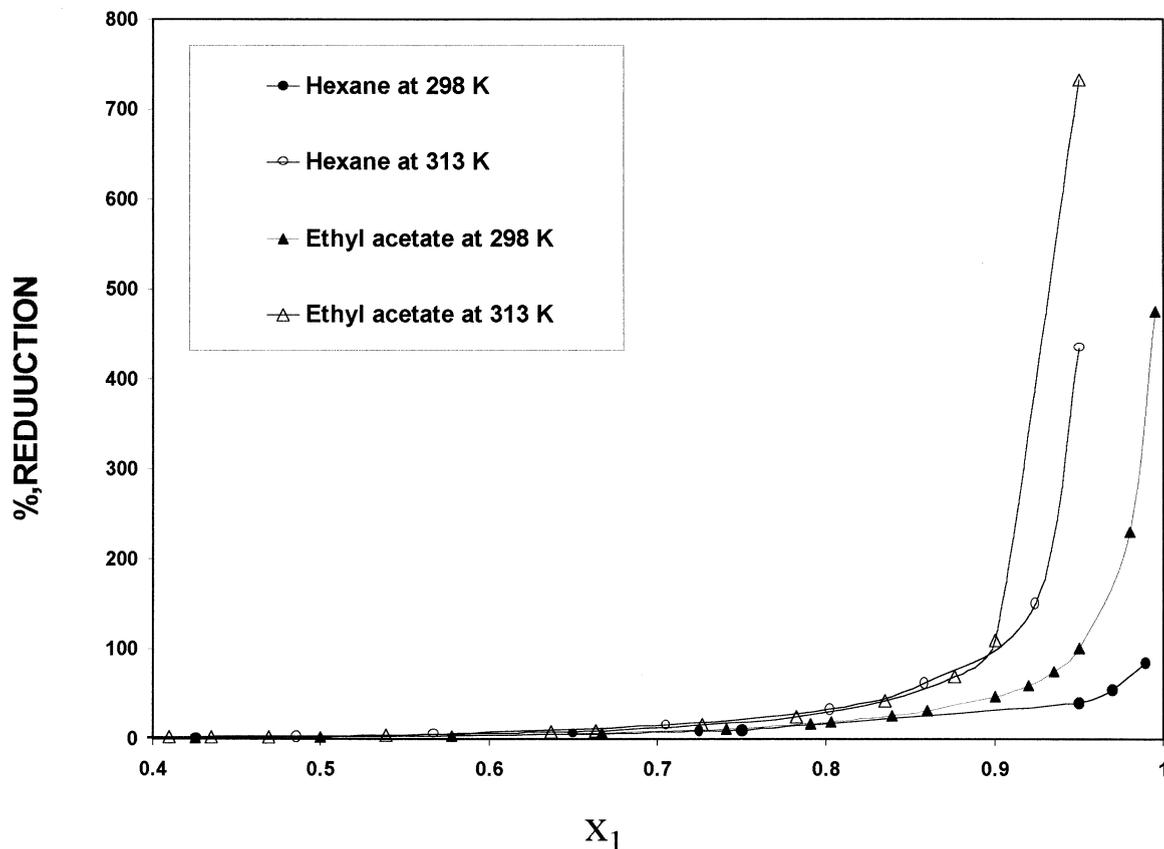


Fig. 10. Comparison of RPMVR of two solvents at two temperatures.

ture as shown in Fig. 6, as a higher pressure is required to retain the same amount of  $\text{CO}_2$  dissolved in the solution at a higher temperature. As a result, there is a greater pressure effect on  $\text{CO}_2$  molecules surrounding the solvent molecule and  $\bar{v}_2$  decreases with temperature.

#### 4.2. Relative molar volume reduction (RMVR)

The values of RMVR and RPMVR have been compared for two solvents, namely hexane and ethyl acetate in Figs. 7 and 8, respectively. As can be seen, both are found to be positive and increase with  $x_1$ . However, RMVR increases with  $x_1$  with no prominent maximum for hexane– $\text{CO}_2$  system, whereas, exhibits a maximum value for ethyl acetate– $\text{CO}_2$  system. It is clear from Figs. 7 and 8 that RMVR is very much less sensitive (com-

pared to RPMVR) in its variation with  $x_1$  at high  $\text{CO}_2$  dissolution and the maximum is not at all prominent. On the contrary, RPMVR increases sharply at high values of  $x_1$  for both systems at 298 K. In other words, RMVR is inadequate for characterization of GASC and it is well understood that the most appropriate parameter is RPMVR for ascertaining the optimum process conditions for GASC.

#### 4.3. Relative partial molar volume reduction (RPMVR)

The partial molar volume reduction,  $(1 - \bar{v}_2/v_2)$  is always found to be positive, which steeply increases at high values of  $x_1$  and directly gives the fraction of crystallization of the dissolved solute. RPMVR has been compared for two solvents in

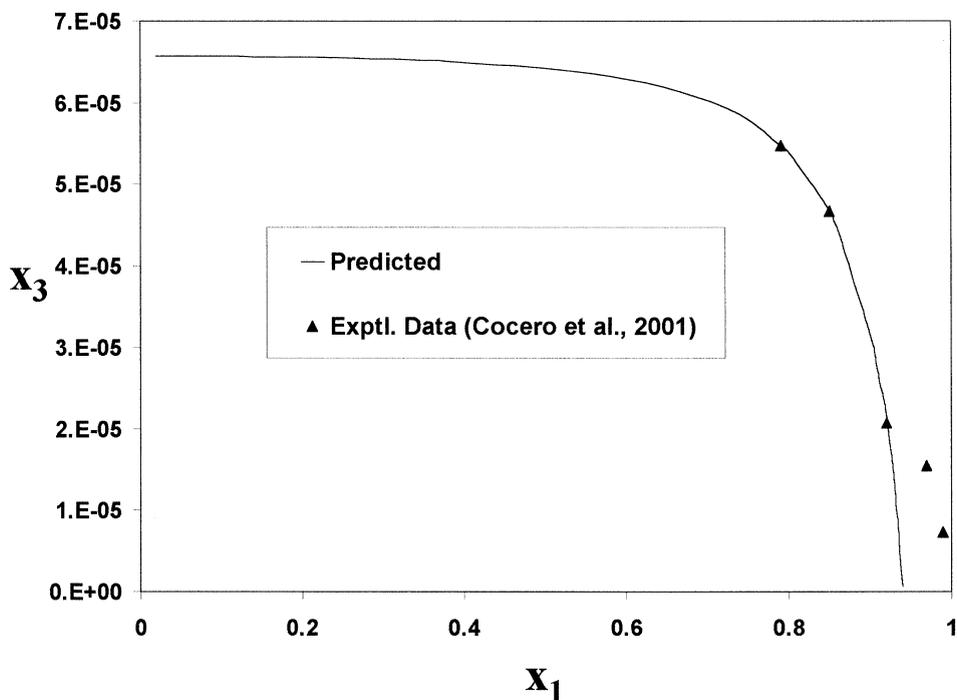


Fig. 11. Predicted solubility of  $\beta$ -carotene in ethyl acetate at 298 K.

Fig. 9 and it can be seen that its variation with  $x_1$  is more sensitive for ethyl acetate than hexane, indicating faster crystallization from ethyl acetate solution than hexane solution. Fig. 10 shows a comparison of RPMVR of two solvents at temperatures 298 and 313 K. It can be clearly understood that the variation of RPMVR with  $x_1$  for hexane at 298 K is much slower than that for ethyl acetate at 313 K. This implies that hexane at 298 K is a preferred solvent for selective (gradual) crystallization needed for purification, whereas ethyl acetate at 313 K is a better solvent for complete (rapid) crystallization needed for micronization. Thus, a supercritical temperature is needed for micronization, whereas a lower (subcritical) temperature is needed for purification. Further, it can be observed that the behavior of RPMVR versus  $x_1$  is able to distinctly distinguish the difference between the temperatures and solvents, which is not possible using the conventional criterion, the total volume expansion, as shown earlier.

#### 4.4. Solute solubility predictions

It is well known that  $\text{CO}_2$  solubility in a solvent increases with pressure and decreases with temperature and the solute solubility in solvent increases with temperature and decreases with  $\text{CO}_2$  dissolution at any temperature or pressure. According to Eq. (7), temperature affects  $x_3$  at ambient pressure (without  $\text{CO}_2$  dissolution) and  $x_1$  affects the ratio,  $\bar{v}_2/v_2$ , in addition to temperature. Therefore,  $x_1$  and temperature are the controlling parameters for the saturated solute solubility in the  $\text{CO}_2$ -solvent system. However, as shown earlier,  $\bar{v}_2$  decreases with temperature resulting  $x_3$  to decrease with temperature. On the other hand,  $x_3$  increases with temperature with or without  $\text{CO}_2$ . So temperature has two opposite effects and due care is taken into consideration in the calculation of  $\bar{v}_2$  and  $x_1$ . Fig. 11 shows the behavior of the predicted solubility of  $\beta$ -carotene in ethyl acetate with  $x_1$  at 298 K which compared well with the experimental data [6]. The marginal deviation at

high values of  $x_1$  may be attributed to experimental uncertainties due to supersaturation or the onset of nucleation. It can be seen that the solute solubility drastically reduces at a high value of  $x_1$ , though it remains almost invariant at lower values of  $x_1$ . The similar trend was reported for naphthalene–toluene and phenanthrene–toluene systems with  $\text{CO}_2$  as antisolvent by Dixon and Johnston [7] based on the experimental data that agreed well with the results predicted by Eq. (7). This trend was also observed for lecithin–hexane system [8].  $x_3$  and  $\bar{v}_2$  have the similar trends for all values of  $x_1$  up to its value at which  $\bar{v}_2$  drastically decreases to 0. The negative values of  $\bar{v}_2$  only indicate that there is clustering of  $\text{CO}_2$  molecules around the solvent molecule causing the solvent molecule to lose its strength for the solute molecule or weakening of solvent–solute bond.

## 5. Conclusions

The gas antisolvent crystallization (GASC) process is particularly useful for purification and micronization of thermo-labile bioactive pharmaceutical solids from their solutions in organic solvents using dense  $\text{CO}_2$  as antisolvent. The solute solubility reduction in the GASC process is directly related to the reduction of the partial molar volume of the solvent with  $\text{CO}_2$  dissolution. At a high  $\text{CO}_2$  dissolution, the solvent molecules are surrounded by the  $\text{CO}_2$  molecules and consequently lose their solvent strength to retain the solid solute in solution. Selection of solvent and process parameters can be based on the new criterion of relative partial molar volume reduction (RPMVR) of the solvent, as it directly gives the fraction of the dissolved solute crystallized. The optimum condition for the GASC process is considered as the  $\text{CO}_2$  dissolution and temperature at which RPMVR drastically increases in the case of micronization and slowly increases in the case of fractional crystallization needed for purifica-

tion. The saturated solute solubility in the liquid phase (on  $\text{CO}_2$ -free basis) has been calculated from its value at ambient pressure times the ratio of the partial molar volumes of the solvent in the  $\text{CO}_2$ -solvent system with and without  $\text{CO}_2$  at the same temperature. The agreement of the predicted solubility with the corresponding experimental data at different values of  $\text{CO}_2$  dissolution, validates the fact that the clustering of  $\text{CO}_2$  molecules around the solvent molecules (or the drastic partial molar volume reduction of the solvent) is in particular responsible for crystallization due to the antisolvent effect. It is also noted that a supercritical temperature is desired for micronization and a subcritical temperature for purification.

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