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Zeta potential as a possible parameter for assessing stability of crystals: Normal and those prepared under electric field.

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Abstract: Crystallization of paracetamol under electrical field brings about changes in physical properties of the crystals. Some of these changes may be desirable from pharmaceutical point of view. In order to examine whether such changes remain stable during storage the stability of surface charge on the crystal , a novel parameter was examined. Crystals obtained without exposure to electric field were included in the study as a control for comparison. It seemed that surface charge measured as zeta potential decayed gradually during storage of both control and experimental crystals which is prepared by exposing to DC electrical field supplied by means of silver and gold electrodes. Correlation studies revealed that there is fair correlation between chemical decay and decay of Zeta potential. Decay of percent content and charge of paracetamol crystals obtained under electrical field was slightly faster. This study indicates the possibility of determination of rate of decay in zeta potential could be a reliable parameter for determining chemical and physical decay. More intense study in this direction could yield fruitful results.
Key words: ENS-crystals, ENG-crystals, Zeta potential and stability studies.

1. Introduction

The crystal habit of a drug is an important variable in pharmaceutical manufacturing. Different crystal forms of a particular drug possess different planes and thus differ not only in their specific surface, but also in their free surface energy. Therefore, they may exhibit different physicomechanical properties such as size, shape, porosity, and compressibility etc. [1]. A crucial factor, particularly in the crystallization processes of pharmaceutical compounds may also be the ability to control the crystal modification and the stability of the product crystals. [2] In the recent years, it has been observed that the electrical field and its nature and strength also substantially influence the properties of crystals [3]. Such exercise has also been employed in medical field for attaining the desired properties of insulin [4, 5]. The alteration in major physical attributes viz. appearance, color, pH, palatability, consistency etc. during storage has been determined for pharmaceutical dosage forms [6, 7]. The present study is an attempt to examine whether the properties desirable from pharmaceutical and medicinal point of view could be obtained by carrying out crystallization under electrical field.

The nature of crystals depends upon the nature of nucleus. Therefore, the exposure to electrical field was made only during nucleation stage. Surface charge as determined by measuring zeta potential The zeta potential (ZP) is a function of the surface charge which develops when any material is placed in a liquid and it

is commonly used to predict and control dispersion stability[8-10] was considered a measure of stability of the crystals.

In the present study both control and ENS and ENG-crystals sample were evaluated for the zeta potential (ZP) which has been employed first time for stability study of crystals and the data obtained is correlated with the degradation rate of paracetamol with respect to time. The physical and chemical stability of any substance depends upon the surface charge which ultimately depends on the status of arrangement of atoms of which it is constituted. Therefore, the changes in ZP with respect to time should be determined in order to predict its stability. Therefore, determination of stability of the pharmaceutical substances by knowing the changes associated with charges with respect to time at specific storage condition along with physical and chemical parameters could serve the purpose more prominently and scientifically. Hence the present study pertained to the crystallization of paracetamol under the influence of DC electric field which may causes the changes in charge content of paracetamol in EN-crystals as compared to control sample. Out of curiosity we wanted to know crystallization of paracetamol under an electric field whether causes the change in the quanta of charges and percent content if any and if so happened how long it is being stable. Therefore, herein we envisage this research work with objective to carry out the stability studies by estimating the changes in ZP with respect to the time and their correlation with percent content of paracetamol.

2. Material and methods

Paracetamol was received as a gift sample from Pharmadia Pharmaceuticals Co. (Nagpur, India). Double distilled water wa s used as a solvent for crystallization.

2.1. Apparatus

The zeta potential was estimated using conventional zeta meter. Two types of electrodes viz. silver and gold were employed and the crystals obtained therewith are termed as ENS (crystal obtained with silver electrodes) and ENG (crystal obtained with gold electrodes). Copper electrodes were discarded because they produced considerable gas. Crystallization cell was used to prepare saturated solution (Figure 1). The electrodes of sliver/gold of dimensions as depicted in the Figure 2 and Figure. 3 was used to obtain the crystals of paracetamol. The stability chamber of Thermolab , India was used to keep the samples for stability studies.
[Insert Figure 1]

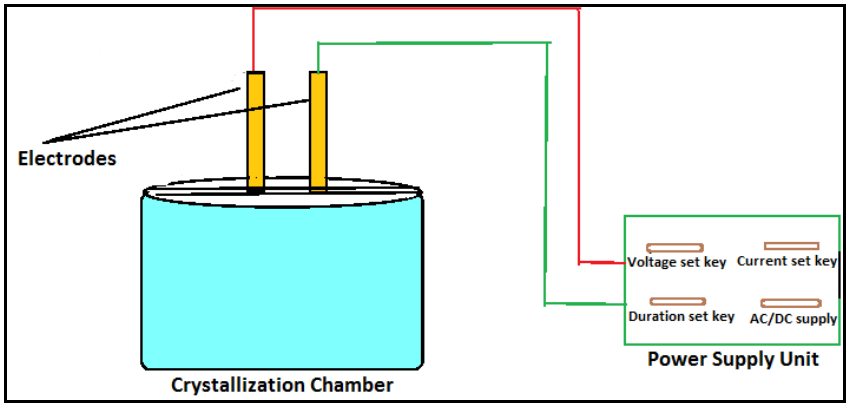

Figure 1 Electro crystallization cell.

2.2. Preparation of paracetamol crystal (Control sample)

The super saturated solution of paracetamol was prepared by dissolving paracetamol (4.5 g) in double distilled water (100 ml) and heated to 70°C in the crystallization cell. Then, it was cooled gradually to 25°C and kept in an incubator for 24 hr. The crystals were obtained by decanting the slurry and dried at 40°C in an oven. The dried crystals were used for further studies.

2.3. Preparation of paracetamol crystals under an electric field

The saturated solution of paracetamol (4.5 g in 100 ml) was prepared in double distilled water in the crystallization cell as shown in Figure 1. Then, the solution was supersaturated by heating at 70°C. The DC

electric field applied using the silver/gold electrodes. The experiments were carried out to investigate the changes occurred in the physical properties of the paracetamol crystals occurred by exposing saturated solution to electrical field under the influence of different variables given below.

ENs and ENg-crystal-I:

ENs and ENg-crystals–I were prepared by supplying DC electric field in the crystallization vessel with the aid of respective electrodes having 2 cm distance between them of potential difference 240 V, current strength 100 mA for 3 min.

ENs and ENg-crystal-II:

ENs and ENg -crystals–II were prepared under DC electric field in the crystallization vessel with the aid of respective electrodes having 2 cm distance between them of potential difference 200 V, current strength 100 mA for 1 min.

ENs and ENg-crystals-III:

ENs/ENg-crystal-III were prepared under DC electric field in the crystallization vessel with the aid of respective electrodes having 1 cm distance between them of potential difference 240 V, current strength 60 mA for 1 min.

ENs and ENg-crystals-IV:

ENs and ENg-crystal-IV is prepared by supplying DC electric field in the crystallization vessel with the aid of respective electrodes having 2 cm distance between them of potential difference 240 V, current strength 100 mA for 1min.

2.4. Stability studies

The control sample and ENs and ENg-crystals-I, II, III and IV were kept in the screw tab glass container of suitable size in the stability chamber at 40 °C with 75 ± 5 % RH for 58 days [11]. The two parameters viz. percent content after 7 day of intervals and zeta potential after 15 day of intervals was determined to study the degradation rate in all the three samples viz., control and ENs and ENg-crystals. The correlation between these two was determined.

2.5. Assay of paracetamol

Percent content (% w/w) of paracetamol was estimated by a method which was adopted with some modification. An accurately weighted quantity (equal to 120 mg) of paracetamol crystals was dissolved in 10 mL of methanol in a 500 mL of volumetric flask and diluted with water, mixed thoroughly. The resultant solution was further diluted to the concentration of 12 µg/mL. Then, the absorbance was recorded at 243 nm using water as the blank. The percent purity of paracetamol was calculated using the calibration curve [12].

2.6. Zeta potential

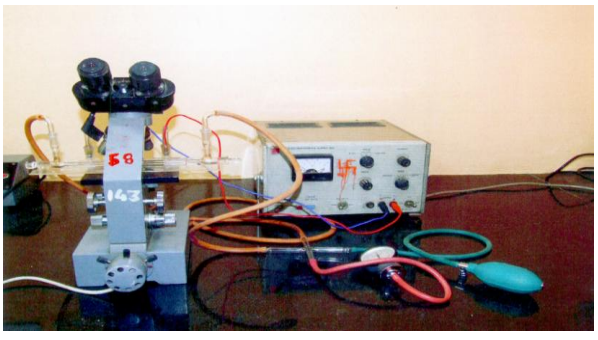

Figure 2 Assembly of conventional zetameter.

The solutions of EN-crystals and control sample [diluted with distilled water (1:50)] to be evaluated for the electrophoresis mobility using conventional zeta meter(figure 2) and the Zp is calculated using equation 1.

$$\xi = \frac{4\pi\eta v}{\varepsilon E} \quad (1)$$

where,
ξ- Zeta Potential (mV)
ε- Dielectric constant of the medium
η- Viscosity of suspending medium [poise]
v- Migration velocity [cm/sec]
E- Potential gradients [volt/cm]

3. Result and discussion

3.1. Physico chemical properties of Control, ENs and ENg crystals

Melting point gives the information about the temperature at which the thermal energy of the particles overcome the intracrystalline forces that hold them in position.[13] The exposure to electric field during nucleation may causes the changes in such forces due to supply of electron from the respective electrodes . hence the effect of electric field supplied by means of silver and gold electrodes during nucleation on melting point is determined . The melting point of ENs and ENg crystals is seems to be do not much alter is shown in table 1. The study of pH and conductance of crystals is an indicative of changes in chemical composition if any that occurred during crystallization under an electric field. The pH and conductance of ENs and ENg crystals also not varied in to greater extent (table 1) which, indicate that the crystallization in electrical field do not causes any modification in chemical composition of the compound. The percent purity of crystals prepared under an influence of electric field also does not seem to be affected.

Table 1 The physical properties of Control sample, ENs and ENg-II, III, IV crystals and I prepared with the aid of silver and gold electrodes

Sr. no	Systems	Physico-chemical properties			
		Melting point (^{0}C)	pH	Conductance (mho)	Assay (%w/w)
1.	Control sample	172^{0}C ±0.921	7.77 ± 0.057	0.60 ± 0.125	99.88%
2.	ENs-crystal-I	172^{0}C ±0.526	7.69 ± 0.098	0.59 ± 2.651	99.91%
3.	ENs-crystal-II	172^{0}C ±0.251	7.77 ± 0.286	0.59 ± 0.763	99.45%
4.	ENs-crystal-III	170^{0}C±0.291	7.59 ± 0.005	0.58 ± 0.245	99.54%
5.	ENs-crystal-IV	172^{0}C±.0.926	7.59 ± 0.005	0.60 ±0.055	99.34%
6.	ENg-crystal-I	172^{0}C ±0.584	7.68 ± 0.435	0.58 ± 1.503	95.03%
7.	ENg-crystal-II	172^{0}C ±1.251	7.68 ± 0.435	0.61 ± 0.973	99.33%
8.	ENg-crystal-III	170^{0}C±0.146	7.70 ± 0.546	0.57 ± 0.925	98.66%
9.	ENg-crystal-IV	172^{0}C± 0.962	7.63 ± 0.554	0.60 ± 0.633	99.12%

***Results are Mean±SD (n=3)**

3.2. ENs-crystals prepared using gold electrodes:

The two parameters viz. percent content and zeta potential were used for stability study of control , ENs and ENg crystals. The 10 % degradation is seemed in both ENs , ENg crystals and controls on 14th day so far as percent content is concerns (table 2 and figure 3). Figure 4 depicts that on 58th day the surface charge (ZP) of both control and ENs-crystals decayed by 25 % and 10 to 14% respectively. The surface charge (ZP) of ENs-crystals decay at the rate slower than control sample exhibits its extended stability(Table 2). The exposure to electric field seemed to enhance the charge stabilization, which revealed that the paracetamol crystals prepared under an electric field provided by means of silver electrodes is more stable.

Table 2 The content% w/w and ZP(mV) of control sample and ENs-crystals-I-IV prepared with the aid of the silver electrodes.

Sr. no	Systems	Parameters	Duration (in days)						
			0	7th	14th	21st	28th	45th	60th
1	Control sample	Content % w/w	100.14	94.35	93.33	79.10	78.33	65.51	52.94
		ZP (mV)	5.949±2.46		5.46±1.94		5.043±2.86		4.51±3.45
2	ENs-crystal-I	Content % w/w	99.48	92.30	91.30	83.71	76.79	62.56	52.69
		ZP(mV)	5.20±2.09		5.608±1.79		4.946±1.29		4.529±2.12
3	ENs-crystal-II	Content % w/w	100.12	94.61	93.71	87.05	72.17	52.82	51.02
		ZP(mV)	4.26±1.65		4.61±2.45		3.92±1.68		3.69±1.45
4	ENs-crystal-III	Content % w/w	99.61	93.84	92.52	76.79	69.87	59.87	49.48
		ZP(mV)	3.84±1.84		3.12±4.21		3.57±2.98		3.62±5.76
5	ENs-crystal-IV	Content % w/w	100.51	94.87	93.58	79.35	75.38	61.92	49.87
		ZP(mV)	4.138±1.902		4.252±2.49		3.167±1.12		4.529±3.67

*Results are Mean±SD (n=40) for ZP

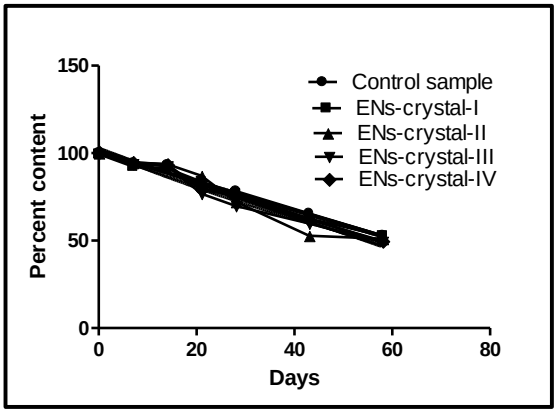

Figure 3 The degradation of percent content of control sample, ENs-crystal-I, ENs-crystal-II, ENs-crystal-III and ENs-crystal-IV.

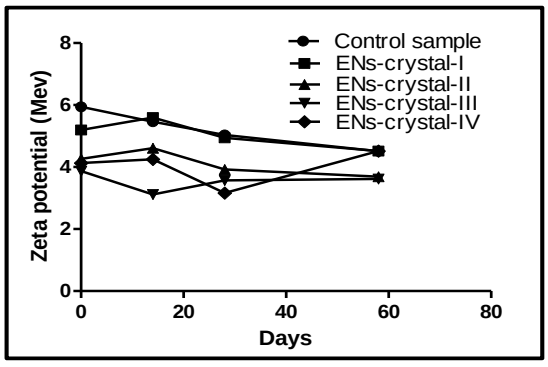

Figure 4 The degradation of zeta potential of control sample, ENs-crystal-I, ENs-crystal-II, ENs-crystal-III and ENs-crystal-IV.

3.3. ENg-crystals prepared using gold electrodes:

Table 3 Thecontent% w/w and ZP(mV)of control sample and ENg-crystals-I-IV prepared with the aid of the gold electrodes.

Sr. no	Parameters	Content % w/w ZP (mV)	Duration (in day)						
			0	7th	14th	21st	28th	45th	60th
1	Control sample	Content % w/w	100.14	94.35	93.33	79.10	78.33	65.51	52.94
		ZP (mV)	5.949±2.46	-	5.46±1.94	-	5.043±2.86	-	4.51±3.45
2	ENg-crystal-I	Content % w/w	99.87	92.56	92.30	88.84	79.35	67.05	53.33
		ZP (mV)	4.105±1.01	-	3.982±2.45	-	3.821±4.87	-	2.529±1.94
3	ENg-crystal-II	Content % w/w	100.76	94.61	93.58	83.97	76.02	60.12	55.89
		ZP (mV)	4.836±0.99	-	4.669±1.16	-	3.981±2.96	-	3.742±1.10
4	ENg-crystal-III	Content % w/w	100.38	94.10	93.58	88.33	79.87	62.94	52.69
		ZP (mV)	5.231±2.64	-	5.546±2.54	-	4.863±1.16	-	4.196±1.46
5	ENg-crystal-IV	Content % w/w	99.61	94.10	93.33	80.64	78.33	65.51	54.23
		ZP (mV)	4.949±1.49	-	4.656±2.48	-	5.043±2.86	-	3.425±3.75

*Results are Mean±SD (n=40) for ZP

The stability of ENG-crystals produced by gold electrodes appear to be superior than that of the ENs-crystals prepared by the silver electrodes so far as stability of percent content is concerns (Table 3 and figure 5). The surface charge (ZP) of ENg-crystals prepared with the aid of gold electrodes decayed at faster rate than that of the control **(**figure 6**)**. The decaying rate of surface charge in ENG crystals is faster than that of control and ENs the reason attributed to this is that the varying nature of electrodes i.e. silver and gold may provides the different environment i.e. flow of electron during exposure to electric field supplied with them at the time of nucleation. It was considered necessary to assess whether the crystals prepared under electrical field undergo instability during storage if it is than at what rate. The stability assessment indicated that no risk of instability since the both control and crystals pareaped under an electric field decay at the same rate . This conclusion is based on the fact that the value of ZP of the substance is the charge on surface of the substance which in turn is the resultant of the equilibrium of various electrical charges operative within the substance. Any change in the zeta potential value would be indicative of the change in intracrystalline charges. There was a slight decay in zeta potential values in both ENs and ENg crystals on 60^{th} day of storage period. This decay was almost similar in all crystals including the control. It therefore seems that ENs and ENg-crystals do not suffer from faster decay of any sort. A marginal decrease in zeta potential (increase in negative charge on surface) could easily be understood because of the deposition of some excessive electrons on surface during crystallization under electrical field which can ultimately altered the reactivity of ENs and ENg crystals with the several environmental factors such as air ,light and moisture during storage. The data was fitted to linear correlation d analysis, which yielded the p, value more than 0.005 and R^2 value which was not close to unity indicating that there is no correlation exit between these two variables viz. paracetamol content %w/w and surface charge(ZP).

This study indicates the possibility of a novel approach of determining decay of charge with respect to the time determining the instability of a substance. The charge on surface of any molecule can be indicative of its nature .In all of these cases surface charge of the substance may be altered because of alteration in the intramolecular forces. By estimating the changes in the surface charge with respect to the time, it can be possible to know the extent of degradation of the substance. Therefore, determination of surface charges and its correlation with the other physical and chemical parameters during the stability study could serve the purpose of knowing stability status.

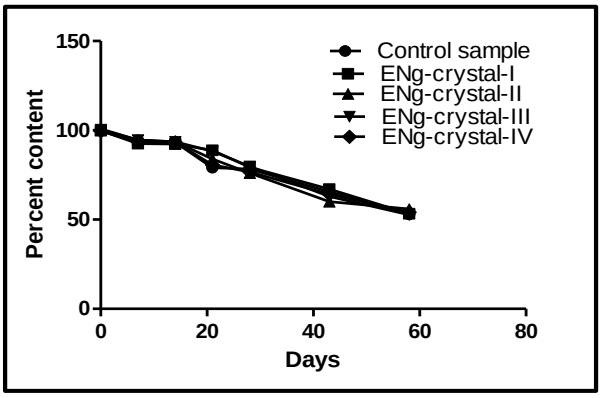

Figure 5 The degradation of percent content of control sample, ENg-crystal-I, ENg-crystal-II, ENg-crystal-III and ENg-crystal-IV.

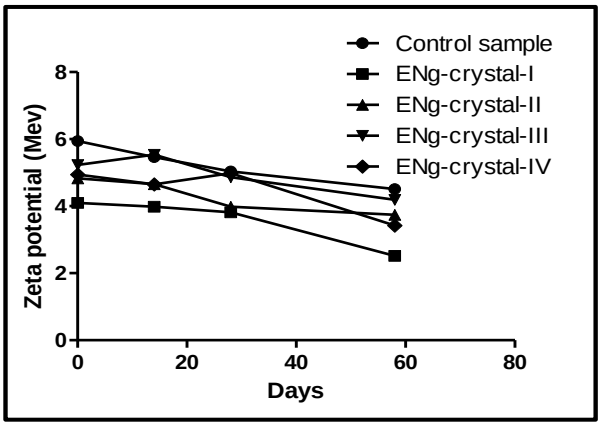

Figure 6 The degradation of zeta potential of control sample, ENg-crystal-I, ENg-crystal-II, ENg-crystal-III and ENg-crystal-IV.

Conclusion

Determination of zeta potential and its correlation with the other parameters can be meaning full for stability studies of a substance.

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