SCANNING ELECTRON MICROSCOPY AS AN ANALYTICAL TOOL FOR PARTICLE SIZE DISTRIBUTION AND ASPECT RATIO ANALYSIS OF CIPROFLOXACIN MUCOADHESIVE POLYMERIC SUSPENSION

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ABSTRACT

Two types of mucoadhesive suspension of Ciprofloxacin were formulated by taking Carbopol934 and Carbopol940 polymers. The morphologies and mechanical properties of the resultant formulations were investigated. The dispersion of particle was observed using Scanning electron microscopy (SEM) techniques. The particle size distribution (PSD) and aspect ratio (AR) of particles in the polymeric suspension were obtained from SEM image analysis. It was found that SEM imaging displays directly the micron level dispersion of particles in polymeric formulation. The technique has significant potential for characterizing such formulation, having some advantages over 'traditional' transmission electron microscopy in terms of generating representative data in a realistic timescale. Considering graphical analysis, the maximum particle size range for formulation containing Cipro and C934 is between 10 and 15 µm but in case of Cipro and C940, it is 5 to 10 µm. In the rod-like particles whose shapes are characterized by the two major and minor lengths of the particle, the length/width ratios are satisfactorily consistent with the aspect ratio value evaluated from the instrumental measurements. Formulation containing Cipro and C934 is more stable because it has lesser standard deviation and co-efficient of variation in aspect ratio analysis. The PSD and AR distribution and degree of dispersion in the formulation give insights into the modification of mechanical properties of the mucoadhesive formulation studied.

Keywords: *Cipro, C934, C940, SEM, PSD, AR, mucoadhesive formulation*

1. INTRODUCTION

The SEM is an analytical tool that uses a focused beam of electrons to form magnified images. SEM image analysis is a promising technique for generating particle distribution profiles as well as surface characteristics with the possibility to visually re-evaluate the data by re-assessing the particle. The technique holds promise for characterization of the size and shape of unknown products (containing particles in polymeric suspension) with relatively wide distribution profiles from the nanometer to the micron range [1]. The particle size distribution (PSD) [2] and aspect ratio (AR) [3, 4] of particles in the polymer phase are obtained from SEM image analysis. The technique has significant potential for characterizing suspension having some advantages over `traditional' microscopy in terms of generating representative data in a realistic timescale. The PSD and AR distribution and degree of dispersion in the suspension give insights even into the stability relating to the modification of mechanical properties, particle-matrix interaction, polymer and drug crystallinity and the overall structure [2, 5, 6] of the suspension. This is also because of WHO guidelines, where it has been mentioned that particle size distribution study is essential for stability of any kind of suspension [7, 8]. It is also known that the reduction of the drug particle size increases the effective surface area and enhances the dissolution, absorption rate and bioavailability of drug. The particle size should be well calculated to avoid the inter-particle attractive forces which lead to instability, lower dissolution and absorption [9].

Ciprofloxacin (Cipro) is a potent second generation fluoroquinolone antibacterial agent [10, 11]. The demand remains for a dosage form that will provide Ciprofloxacin at a sustained, constant level in solution in the acidic and basic p^H conditions of the gastrointestinal tract over the full dosage period. Taking into consideration of the above factors, mucoadhesive suspensions of ciprofloxacin were prepared by using two grades of carbopol polymer i.e., Carbopol934 (C934) and Carbopol940 (C940). Both C934 and C940 consist of chains of polyacrylic acid and they only differ by the cross linking agents like allyl ethers of Sucrose in C934 and allyl ethers of Pentaerythritol in

C940. Carbopol polymers are p^H sensitive [12, 13], environmentally responsive polymers or considered as smart gels [14, 15]. Along with this other additives are used to stabilize this mucoadhesive suspension.

Since range is the simplest method of studying dispersion, different ranges of length of particles along with their frequencies have been considered [16, 17]. In this study, SEM imaging was used to display the particle dispersion in two types of polymeric mucoadhesive suspensions.

2. MATERIALS

The following materials were used: Ciprofloxacin Hydrochloride was obtained from Dr. Reddy's Lab, Hyderabad, India, as a gift sample. Carbopol934, Carbopol940, Pluronic F68 and Soya lecithin were purchased from Himedia Laboratories Pvt. Ltd., India. Citric acid, Sodium citrate, Glycerol, Mehtyl praraben, Propyl paraben, Sorbitol solution I.P. and Sucrose were kindly supplied by Cosmo Chem. Laboratory, India. Ultra pure water was obtained from a Millipore Milli-Q UV water filtration system.

3. METHODS

3.1 Preparation of Formulation

3.1.1 Preparation of Bulk A

In a beaker 6ml water was taken and heated up to 80^0C. Sucrose (10gm) was added under continuous stirring. The temperature was monitored in such a way so that it should not fall bellow 70^0C till the sucrose completely dissolved. The prepared syrup was cooled properly to room temperature and kept overnight. Syrup was filtered using 120 mesh nylon cloth.

3.1.2 Preparation of Bulk B

5ml of Ultra pure water was taken in a beaker to which 1.8ml of sorbitol solution and 0.2ml glycerin were added. The mixture was stirred properly. To this solution pluronic-F-68 (5%), soya lecithin (1%) and carbopol940 or carbopol934 (5%) in w/w of drug were added with continuous stirring.

3.1.3 Preparation of Mucoadhesive Suspension and Ultrasonication

5ml of water was taken in another beaker to which 1.25gm of ciprofloxacin HCl was added with continuous stirring. To the drug suspension, the Bulk B and Bulk A were added with continuous stirring. Methyl paraben sodium (0.015%w/v) and Propyl paraben sodium (0.08%w/v) were used as preservative. The volume was made up to 25ml by Ultra pure water. The p^H was adjusted by adding citrate buffer (0.75M) to p^H5.

Homogenization was carried out for at least 15min by ULTRASONIC HOMOZENIZER LABSONIC^R M (SARTORIUS) having operating frequency 30KHZ and line voltage 230V/50HZ. The probe used for ultrasonication was made up of Titanium having diameter 7mm and length 80 mm. The setting knob "cycle" was adjusted to 0.8; indicating sound was emitted for 0.8s and paused for 0.2s. In this manner, we could expose our sample with 100% amplitude, while reducing the heating effect to 80%. This LABSONIC^{R}M generates longitudinal mechanical vibrations with a frequency of 30,000 oscillations/s (30KHZ). The probes bolted to the sound transducer are made of high-strength Titanium alloys, built as $\lambda/2$ oscillators. It amplifies the vertical oscillation, and transfer the ultrasonic energy via its front surface with extremely high power density into the sample that is to be subjected to ultrasonic waves. Here, stress applied was sound wave and mild rise in temperature of the sample occurred during ultrasonication.

3.2 SEM Analysis

The Mucoadhesive suspension was sprayed on to an aluminum slip with the aid of an atomizer. The fine droplets were dried overnight and it was used for SEM analysis [10]. The samples were given a conductive coating (using Pt, of about $600A^0$ thick) using sputter ion coater and examined with scanning electron microscope (JEOL JSM-6480LV) equipped with a backscattered electron detector for imaging and EDXA for elemental analysis. In this method, a focused electron beam is scanned over the sample in parallel lines. The electrons interact with the sample, producing an array of secondary effects, such as back-scattering, that can be detected and converted into an image.

The image can then be digitalized and presented to an image analyzer, which uses complex algorithms to identify individual particles and record detailed information about their morphology (Figure 1 and 2).

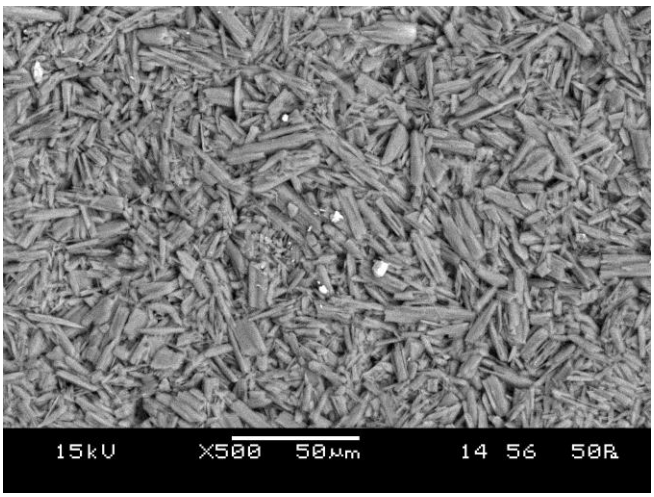

Figure 1. SEM of Cipro and C934

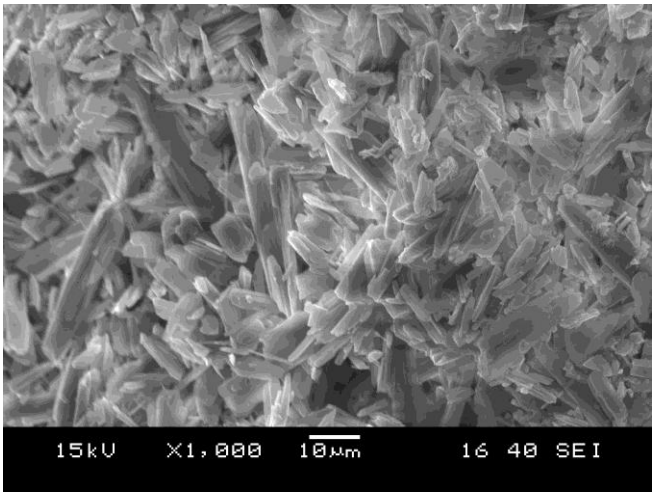

Figure 2. SEM of Cipro and C940

3.2.1 methods of particle size and aspect ratio determination

The particle size can be determined with a program such as Image Tool or annotate either automatically or manually. Here, manual determination is preferred, because sometimes the particle boundaries are indistinct, and the software may interpret them incorrectly. The PSDs reflect the statistical result from all sections for each sample. As these are rod like particles the aspect ratios of rod-like particles are evaluated by comparing the particle size distribution data derived from SEM analysis following the techniques described by Jennings and Parslow (1988) [5]. Length/width ratios are satisfactorily determined the aspect ratio value.

4. RESULTS

Table 1 shows PSD analysis of samples containing Cipro with C934 and Cipro with C940. In that table different ranges of length of particles of both the samples along with their frequencies have been mentioned. While within 0-5 µm range no particle was found, maximum number of particles was observed within 10-15 µm range when a sample containing Cipro with C934 was used. In case of a sample containing Cipro and C940, minimum number of particles was found within 0-5 µm range and maximum number of particles was detected within 5-10 µm range.

In both the formulation, the particle aspect ratios (length/width) were calculated (Table-2). While in case of formulation containing Cipro and C934, maximum aspect ratio frequency was found from 4 to 6, in case of Cipro and C940, it was between 2 and 4. From these data (PSD and aspect ratio analysis) arithmetic means, standard deviation and co-efficient of variation have been calculated using standard formula [16].

Mean ($\bar{A}$) = $A + (\Sigma fd / N) \times i$
Standard Deviation = $\sigma = \sqrt{[(\Sigma fd^2 / N) - (\Sigma fd / N) 2]} \times i$
Coefficient of Variation or C.V. = (Standard Deviation / Mean) × 100

Table 1. PSD analysis of samples

a) CIPRO AND C934.									
LENGTH (μm)	f	c.f	m.p. m	(m –A) / d (m-17.5)/d	fd	Ā	fd^2	σ	C.V.
0-5	0	0	2.5	-3	0		0		
5-10	14	14	7.5	-2	-28		56		
10-15	26	40	12.5	-1	-26		26		
15-20	8	48	17.5	0	0	13.24	0	5.035	38%
20-25	4	52	22.5	1	4		4		
25-30	2	54	27.5	2	4		8		
	N=54				Σ fd= -46		Σ fd^2= 94		
b) CIPRO AND C940.									
LENGTH (μm)	f	c.f	m.p. m	(m –A) / d (m-17.5)/d	fd	Ā	fd^2	σ	C.V.
0-5	1	1	2.5	-2	-2		4		
5-10	28	29	7.5	-1	-28		28		
10-15	18	47	12.5	0	0		0		
15-20	4	51	17.5	1	4	9.95	4	3.34	33.56 %
20-25	0	51	22.5	2	0		0		
25-30	0	51	27.5	3	0		0		
	N=51				Σ fd= -26		Σ fd^2= 36		

Table 2. Aspect ratio analysis of samples

a) CIPRO AND C934.									
A.R. (l/d)	f	c.f	m.p. m	(m –A) / d (m-17.5)/d	fd	Ā	fd^2	σ	C.V.
0-2	4	4	1	-3	-12		36		
2-4	15	19	3	-2	-30		60		
4-6	19	38	5	-1	-19		19		
6-8	8	46	7	0	0		0		
8-10	6	52	9	1	6	5.35	6	2.67	49.9%
10-12	1	53	11	2	2		4		
12-14	0	53	13	3	0		0		
14-16	1	54	15	4	4		16		
	N=54				Σ fd= -49		Σ fd^2= 141		
b) CIPRO AND C940.									
A.R. (l/d)	F	c.f	m.p. m	(m –A) / d (m-17.5)/d	fd	Ā	fd^2	σ	C.V.
0-2	6	6	1	-4	-24		96		
2-4	19	25	3	-3	-57		171		
4-6	15	40	5	-2	-30		60		
6-8	6	46	7	-1	-6		6		
8-10	2	48	9	0	0	4.76	0	3.28	68.8 %
10-12	1	49	11	1	1		1		
12-14	0	49	13	2	0		0		
14-16	0	49	15	3	0		0		
16-18	2	51	17	4	8		32		
	N=51				Σ fd= -108		Σ fd^2= 366		

The frequencies and cumulative frequencies of particle size distribution (PSD) of Cipro with C934 and Cipro with C940 are presented graphically by taking particle size ranges along X-axis, the frequencies of respective ranges on Y- axis the cumulative frequencies on the ŸŸ – axis (Table 1, Figure 3, 4). The PSDs vs frequencies are plotted as histogram and PSDs vs cumulative frequencies are plotted as curves. Y- axis represents the frequencies of PSD ranges which constitutes the height of its rectangles. We get a series of rectangles each having a class interval distance as its width. The area of the histogram represents the total frequency as distributed throughout the classes.

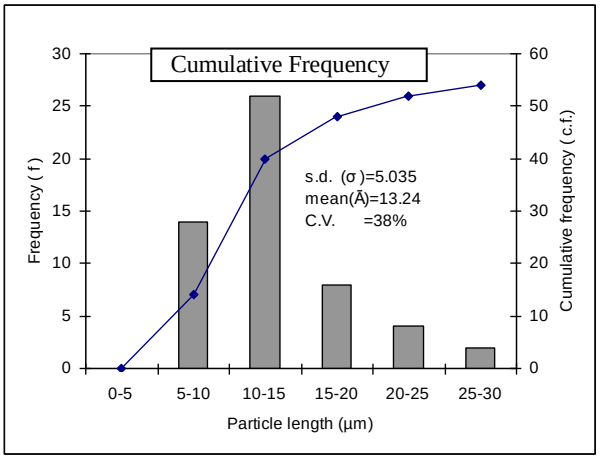

Figure 3. PSD Analysis of Cipro and C934

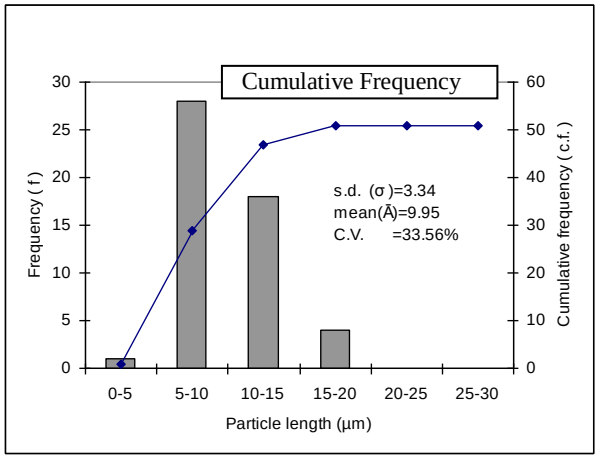

Figure 4. PSD Analysis of Cipro and C940

Similarly the Aspect ratio ranges vs frequencies and cumulative frequencies of both the formulation are plotted (Table 2, Figure 5, 6).

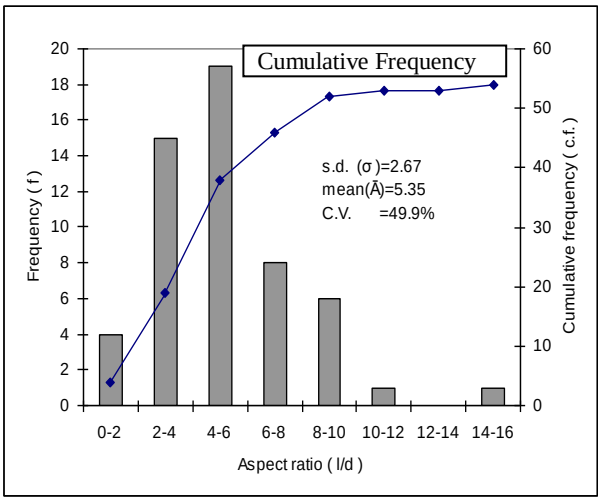

Figure 5. AR Analysis of Cipro and C934

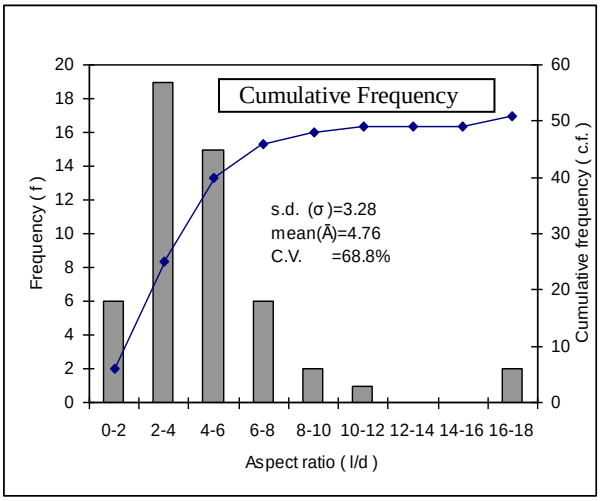

Figure 6. AR Analysis of Cipro and C940

5. DISCUSSION

Since an average is a single value representing a group of observation, it must be properly interpreted, otherwise, there is every possibility of jumping to wrong conclusion. Sometimes the average may give a value that does not exist in the data .So the measures of dispersion help us in studying important characteristics of a distribution. According to Simpson and Kafka "The measurement of the scatterness of the mass of figures in a series about an average is called measure of variation or dispersion" [16]. The standard deviation measures the absolute dispersion

(or variability of a distribution; the greater amount of dispersion or variability). The greater the standard deviation, greater will be the magnitude of deviations of the values from their mean. While a small standard deviation indicates a high degree of uniformity of the observations as well as homogeneity of a series, a large standard deviation means just opposite. Thus, if we have two or more comparable series with identical or nearly identical means, it is the distribution with smallest standard deviation that has the most representative mean.
The series (or group), for which the co- efficient of variation is greater, is said to be more variable, and conversely less consistent, uniform, stable or homogeneous. On the other hand, the series, for which co- efficient of variation is less, said to be less variable, and more consistent, uniform, stable and homogeneous. From particle size distribution study it has been found that in both the formulations maximum particle size is within 5 to 15 µm, which is pharmaceutically acceptable [17] (Table 1). Considering graphical analysis, the maximum particle size range for formulation containing Cipro and C934 is between 10 and 15 µm but in the other case (Cipro and C940) it is 5 to 10 µm (Fig.3and 4). As these are rod like particles, the aspect ratio has been calculated. From the statistical interpretation it has been found that aspect ratios in formulation containing Cipro and C934 is more homogeneous, consistent and stable than formulation containing Cipro and C940, as former formulation having particles with lesser standard deviation and co-efficient of variation (Table 2, Figure 5,6). While the formulation containing Cipro and C934 has mean particle size and aspect ratio of 13.24 µm and 5.35 respectively, formulation containing Cipro and C940 shows PSD and AR values as 9.95 µm and 4.76 respectively. The results show a correlation between the particle size and particle shape and stability properties, giving confidence in the usefulness of SEM for characterizing this type of formulations [18,19].

Based on the above-mentioned discussion, it can be suggested that the mechanical properties of formulation may be predicted very well with the information from PSD and AR distributions derived from SEM images. It proves the ability of SEM to give reliable and useful results for the polymeric suspension, and generate data for in situ particle properties which are difficult to obtain in other ways.

6. CONCLUSIONS

The morphologies and mechanical properties of mucoadhesive suspension having two polymeric composites show SEM sectioning and imaging can allow direct measurement of PSD and AR of particles embedded in polymeric suspension. The micron level dispersion of the particles can be directly accessible. The SEM-derived information correlated well with the mechanical properties of formulation studied here. From the above SEM image analysis it has been concluded that the formulation containing Cipro and C940 having better bioavailability and penetration capacity, as maximum particles having aspect ratio of 2 to 4 which is smaller than that of formulation containing Cipro and C934 [9]. On the other hand, when pharmaceutical stability is considered, formulation like Cipro and C934 is more stable because it has lesser standard deviation and lesser co-efficient of variation in aspect ratio analysis, so the particles are more uniformly dispersed. Further work should be carried out to demonstrate whether SEM imaging is actually a suitable approach for observing the PSD and AR distributions and degree of dispersion of micron size particles.

7. REFERENCES

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