The Particle Size Evaluation of Parenteral Suspensions of Chloramphenicol and Correlation of Particle Size to Plasma Levels\(^1\)

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The method of particle size evaluation of chloramphenicol suspensions with the Coulter counter has been studied. Using the salt solution, which was supersaturated with chloramphenicol and contained a small amount of polyvinylpyrrolidone to attain a wide range of supersaturation of chloramphenicol, as a diluent, the particle size analysis of chloramphenicol suspensions can be made reproducibly in the size range between 0.7 and 2.0 \(\mu\). Chloramphenicol suspensions containing different average size particles were administered intramuscularly to rabbits, and it was found that the peak plasma levels obtained were directly related to the reciprocals of the average particle diameters measured by this method.

It has been well known that the particle size of poorly soluble drugs has a striking influence on the bioavailability. In parenteral dosage forms, drug particle size is considered to be one of the important factors for the drug availability as well as viscosity and flocculation.

In the case of the pharmaceutical development of chloramphenicol suspension for parenteral use, we found that the smaller particle suspensions gave the higher blood levels than the larger particle suspensions, when the chloramphenicol suspensions of various particle sizes were administered intramuscularly to rabbits. However, we encountered the difficulty of analyzing particle size and particle size distribution of chloramphenicol because particle size was very small. Although a number of method have been proposed for particle size analysis such as light scattering, sedimentation, and microscopy, these methods are of their limited utility. For example, light scattering technique is much more complicated and microscopic technique is time-consuming and not sensitive enough to analyze particles having a smaller than micron size. Currently, the Coulter counter has been introduced as a very useful instrument for size analysis of particles in emulsions and suspensions. The Coulter counter has been applied mostly to characterize the dispersions of insoluble solids or oils and little is known of the measurement of slightly soluble particles,\(^3\) since even particles having a very low solubility dissolve in the diluent medium to some extent during the process of counting and this results in reduction of particle size. Paradoxically, the Coulter counter may be applicable to the size analysis of relatively water soluble particles if dissolution of particles can be inhibited.

Based on this consideration, we investigated the particle size analysis of chloramphenicol suspensions by means of the Coulter counter successfully. And the effects of the chloramphenicol particle sizes on the blood levels by the intramuscular administration were studied.

**Experimental**

**Particle Size Determination**—The suspensions tested were four commercial lots of chloramphenicol suspensions (25\% w/v) produced by four different pharmaceutical manufactures.

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1. This work was presented at the 93rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1973.
2. Location: 2-17-85, Juso-honmachi, Yodogawa-ku, Osaka, 532, Japan.
A Coulter counter model A with 30 μ orifice tube was used to determine the particle size distributions. The instrument was calibrated with mono-disperse latices (Dow Chemical Co.) of 0.706, 1.011, and 1.947 μ in diameter, respectively. An excellent agreement of calibration factors for three standard particles was obtained.

The electrolyte solution used for the dilution of samples consisted of 1.0% NaCl, 0.4% chloramphenicol and 0.05% polyvinylpyrrolidone (PVP40). Procedures for preparing the solution were as follows: 10 g of NaCl, 4 g of chloramphenicol and 0.5 g of PVP were completely dissolved in 1 liter of distilled water at about 70° with constant stirring. After cooling to room temperature (20—25°), the solution was filtrated through a 0.22 μ Millipore filter and the resulting filtrate was used as the diluent. Though the diluent was stable for at least 24 hr, the diluent should be made freshly every day before use, since the background counts of the diluent gradually increased as time passed. The size analysis was carried out in the size range between 0.7 and 2.0 μ at room temperature according to the procedures described in the Coulter counter manual. Samples were diluted with the electrolyte solution mentioned above by a factor of 10⁵ for the suspensions A and B, of 5 × 10⁴ for the suspension C and of 10⁴ for the suspension D.

Deflocculation experiment was carried out by homogenization of the suspensions using a Potter-Elvehjem homogenizer having a plastic pestle.

Animal Experiment—Male albino rabbits weighing around 3 kg were used in the absorption studies. The rabbit was fixed on the operation table in supine position and 1 ml of the suspension corresponding to 250 mg of chloramphenicol was injected into femoral muscle. Blood samples were withdrawn by cardiac puncture at appropriate time intervals after the injection. Chloramphenicol in the plasma was determined colorimetrically by a modification of Negoro's method.⁵ A 1 ml-aliquot of each plasma sample was diluted with 5 ml of distilled water and then 2 ml of 0.3 n Ba(OH)₂ solution and 2 ml of 5% ZnSO₄ solution were added to precipitate the proteins. After centrifuging, 3 ml of the supernatant was pipetted into graduated test tubes. To the tubes 2 ml of 2 n HCl and approximately 50 mg of Zn powder were added. The tubes were then heated in a boiling water bath for 1 hr. After cooling rapidly to room temperature, volume were adjusted to 5 ml with 1 n HCl. The samples were mixed thoroughly, the zinc was allowed to settle and 4 ml portions of the clear supernatant solution were transferred to test tubes. To the tubes 0.5 ml of 0.1% NaNO₃ solution was added and the contents were mixed and allowed to stand for 5 min. Then, 0.5 ml of 0.5% NH₄SO₄-NH₄Cl solution was added to decompose excess HNO₃. After the solution has stood 5 min, 0.5 ml of 0.2% 1-β-dihydminoethylamine)naphthalene solution⁶ was added. All the tubes were then placed in a water bath at 37° for 1 hr. The absorbance is measured relative to a reagent blank at 530 mμ.

Results and Discussion

Proposed Analytical Method

Prior to size analysis, the samples of chloramphenicol suspensions should be diluted with the electrolyte solution by a factor of up to 10⁵ in order to provide a favourable concentration for the instrument. However, when the samples were diluted only with 1.0% sodium chloride solution, the counts at a fixed threshold value decreased gradually as the measurement was repeated and this tendency became remarkable especially at a smaller size range. This phenomenon suggested that chloramphenicol particles were dissolving during not only the dilution process but the counting process, in spite of its relatively low water solubility. Therefore, an attempt to prevent the particles from dissolving into the diluent was undertaken and the following three methods were conceivable:

1) The electrolyte solution was previously saturated with chloramphenicol.

2) The electrolyte solution was previously saturated with chloramphenicol and a cellulose bag containing the suspension of micronized chloramphenicol was immersed in the diluent during the measurement.

3) The electrolyte solution was supersaturated with chloramphenicol and a small amount of PVP, which is known as an inhibitor of nucleation and crystal growth of chloramphenicol, was added to the solution.

In order to examine the reliability of these methods, the particle size of chloramphenicol suspensions was analyzed by employing each of the three methods. When the particle size

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4) PVP K-30, Wako Pure Chemical Industries, Ltd., Osaka, Japan.
5) H. Negoro, Yakugaku Zasshi, 70, 669 (1950).
analysis was carried out with the method 1, a decrease in counts was still observed, otherwise the orifice of the tube was clogged with needle-shaped microcrystals of chloramphenicol. These phenomena can be attributed to the solubility variation of chloramphenicol with temperature, since the solubility of chloramphenicol is highly sensitive to temperature and it is well known that crystallization of chloramphenicol readily occurs from its supersaturated solution when temperature falls down to a minor extent.

Application of the method 2 also gave a result similar to that of the method 1. It was evident therefore that the cellophane membrane was not permeable enough to allow a ready passage of the chloramphenicol molecules for maintaining the state of saturation if the concentration of chloramphenicol in the outer solution was varied with temperature.

On the contrary, the method 3, that is, an addition of PVP to the supersaturated solution of chloramphenicol was found to be effective in preventing the dissolution or the crystal growth of chloramphenicol particles. Consequently, it was confirmed that 0.4% of chloramphenicol was suitable for making up its supersaturated solution and an addition of 0.05% PVP was enough to maintain the supersaturation of chloramphenicol when the measurement was carried out at 20—25°. The results of the experiment with this diluent system proved that a variation in the observed counts was almost negligible even if the measurement was repeated and the method now proposed was sufficiently sensitive and reproducible for the application of the size analysis of various chloramphenicol suspensions.

**Estimation of Particle Size Distribution of Chloramphenicol Suspensions**

The size distribution curves of four commercial chloramphenicol suspensions from different manufactures are given in Fig. 1. The curves in Fig. 1 represent the differential count curves for the suspensions corresponding to those which are diluted by a factor of 10⁵ for the convenience of easy comparison. For the two finer samples A and B, the distribution curves were not in their entireties, because particles smaller than approximately 0.7 μ in diameter could not be counted owing to the practical limit of the instrument. Plots of the data of the two other coarse samples C and D on the log-normal probability paper gave the straight lines. The samples A and B also showed the same tendencies as those of C and D when their data were treated similarly. This indicates that the particle size distribution of all these samples may be assumed to have a log-normal distribution.

Although several methods have been proposed for digitalization of particle size data, the mean diameter and the standard

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean diameter (μ)</th>
<th>Standard deviation (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.85</td>
<td>0.18</td>
</tr>
<tr>
<td>B</td>
<td>0.95</td>
<td>0.25</td>
</tr>
<tr>
<td>C</td>
<td>1.10</td>
<td>0.38</td>
</tr>
<tr>
<td>D</td>
<td>1.65</td>
<td>0.55</td>
</tr>
</tbody>
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Fig. 1. Size Distribution Curves of Four Commercial Chloramphenicol Suspensions
deviation were used for characterization of the size distribution of chloramphenicol suspensions in the present study. Table I lists the results with these four suspensions.

In order to examine whether the size distribution curves in Fig. 1 would be represented by the primary particles or not, the size distribution of the sample A was measured by varying the concentration of particles. It was found that the peak height of curves was lowered proportionally as the concentration of particles was decreased as shown in Fig. 2. The results of Edmundson's correction method\(^7\) also showed that the presence of the coincidence error was negligible. From these facts, the size distribution curves shown in Fig. 1 were proved to be those of primary particles. Additionally, a microscopic observation of these suspensions supported the above conclusion.

![Fig. 2. Relationship between Shape of Distribution Curve and Particle Concentration](image)

Key: Relative particle concentration is (a) 2.0, (b) 1.0, and (c) 0.5 respectively.

![Fig. 3. Deaggregation of Particles with Homogenization](image)

When a suspension is allowed to stand for a relatively long period of time, a change in particle size sometimes occurs and this can cause not only physico-chemical changes such as creaming or sedimentation of dispersed particles but also changes in drug availability.

It has been known that the Coulter counter makes it feasible to determine the floc-size of suspensions.\(^8\) In the case of the chloramphenicol suspensions, an increase in the average particle size was observed when the suspension was stored at 40° and the average particle size became larger with time of standing. This phenomenon was probably due to either the growth of crystals or the flocculation of particles. To demonstrate a cause of the increase in the average particle size, the suspension which was kept at 40° for an appropriate period of time was homogenized in a Potter-Elvehjem homogenizer. A remarkable increase in the counts at the smaller size range was observed after a single treatment as shown in Fig. 3. Less than five revolutions in the homogenizer were necessary to recover the initial size distribution curve and no further change of size distribution curve was observed by another repeat of treatment. On the contrary, only a slight change in the average size was found with the suspension containing coarse primary particles of chloramphenicol. The present experiments suggest that the flocculation of particles may take place in the chloramphenicol suspensions during storage and the crystal growth is negligible within this period of time.

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Effect of Particle Size on Plasma Level

The studies of biopharmaceutical aspect have become desirable in addition to the physicochemical aspect for the development of pharmaceutical products. An relation between the particle sizes obtained by the Coulter counter method and the plasma levels of chloramphenicol after intramuscular injections to rabbits was studied. Many studies on the influence of particle size in drug absorption have been published and Fincher\(^9\) has reviewed these works. However, little information is available on the relation between particle size and drug absorption in parenteral suspensions, although Buckwalter and Dickinson\(^{10}\) and Miller and Fincher\(^{11}\) have studied particle-size influences on drug availability. Their results were qualitative, probably because the particle size distributions of samples were so wide that the dissolution rate of drugs was not simply related to a function of the average diameter.

The mean blood levels were plotted as a function of time after intramuscular administrations of four chloramphenicol samples in Fig. 4. A minimum of 10 rabbits was used for each sample. It is obvious from Fig. 4 that the drug was absorbed from the larger particle suspensions more slowly and available to a lesser extent than the smaller particle suspensions.

![Graph](image)

Fig. 4. Mean Plasma Concentration Curves following the Administration of Four Commercial Chloramphenicol Suspensions

key: same as in Fig. 1

Sample B contained some smaller particles than that of sample A, even though the average particle size of sample B was larger than that of sample A. This difference could not be detected by the particle size analysis using the Coulter counter because of the sensitivity limit of the instrument. In fact, the absorption rate of sample B which was larger than that of sample A as shown in Fig. 4 may be explained by the difference in the amount of small particles between sample A and B. The fraction of small particles, however, is not so large that the plasma level may gradually go down to normal level.

It is well known that the viscosity of suspensions depends on the particle size (distribution), even if the volume fraction of particles is held constant. With intramuscular dosage forms the drug absorption may be affected by the viscosity of suspensions. The viscosity effect on the drug absorption was examined by administration of the suspensions which contained the same lot of particles and have the same formulation except the amount of carboxymethylcellulose. The results of this test confirmed that the effect of the viscosity on the drug absorption could be negligible in this case.

If there is a relation between the drug absorption and the particle size of suspensions injected, the rate of absorption must be dependent upon the dissolution rate of particles. Since the specific surface area is proportional to the reciprocal diameter and since the dissolution rate of particles is dependent upon the specific surface area, the rate of absorption would be a function of the reciprocal diameter of the samples. The peak plasma level was used as a comparative measure of the absorption rates of four samples. A good correlation was found between peak plasma level and reciprocal diameter of the samples as shown in Fig. 5. This suggests that the absorption of chloramphenicol after intramuscular injection might be controlled by the dissolution rate of chloramphenicol particles in the muscle tissue. Also, a meaningful correlation between peak plasma level and reciprocal diameter of samples could be found because the chloramphenicol suspensions tested have the relatively narrow particle size distributions.