removed from each tube over 2 hr and examined by phase contrast microscopy at a magnification of 1000× for spheroplasts or abnormal cell shapes.

Regression Analysis of Antibacterial Activities—This analysis was carried out by the method of least squares[6].

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[6] Using the Statistical Package for the Social Sciences, version 600, and the CDC 6400 computer at the Dalhousie University Computer Centre.

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Ball Milling as a Measure of Crushing Strength of Granules

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Abstract □ When granules are milled in a ball mill, the size decrease follows a modification of Kick's law. The Briggsian decay constant, here denoted as the attritional crushing strength, shows correlation with the Harwood–Pilpel crushing strength. Both crushing strengths show a correlation with the amount of granulating agent (povidone) added to the granulation, *i.e.*, the more povidone added, the harder the granule.

Keyphrases □ Granules—ball milling as a measure of crushing strength □ Hardness, granule—ball milling as a measure of crushing strength

The measurement of granule hardness is an important parameter in pharmacy research and quality control. In the simplest method, that of Harwood and Pilpel (1, 2), a granule is placed on a supported plane surface and then brought in contact with the flat bottom of a balance pan. Metal shot is placed in the pan until the granule breaks, and the weight of the shot is then determined. This weight is denoted as the crushing strength of the granule.

It was shown previously (3, 4) that the crushing strength obtained by the Harwood–Pilpel method, when used on granules, requires the averaging of many granules because the observed crushing strength is a function of the way in which the granule is positioned under the pan. The method is fully functional once this fact is realized. An average of 20 measurements suffices for good characterization but is somewhat time consuming. Another disadvantage of the Harwood–Pilpel method is that the crushing strength of granules finer than 40 mesh is difficult (impossible) to measure.

Therefore, an alternative method, equally convenient but applicable to all particle sizes, is desirable. This paper describes such a procedure and shows the correlation between it and the method of Harwood and Pilpel (1) for povidone granulations. Its applicability to other granulations (exemplified by a gelatin granulation) is also discussed.

EXPERIMENTAL

A 6.7-cm i.d. × 13.5-cm long ball mill[1] was used. Granulations were made of the compositions shown in Table I; the povidone was added in a 2-propanol solution to a lactose and corn starch mixture. The dried granules were sized, and the 14/20-mesh fraction was used for further study. Granule hardness was determined by the method of Harwood and Pilpel (1, 3, 4). The lactose used had a particle size of 10–20 μm.

In addition, the following method of determining the crushing strength

[1] Fisher Scientific, Pittsburgh, PA 15219.

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Table I—Formulas of the Granulations Used for Crushing Strength Studies

Component	Formula I	II	III	IV	V	VI	VII	VIII
Lactose USP	1.75 kg	1.75 kg	1.75 kg	1.75 kg	1.75 kg	1.75 kg	1.75 kg	1.75 kg
Corn starch USP	0.75 kg	0.75 kg	0.75 kg	0.75 kg	0.75 kg	0.75 kg	0.75 kg	0.75 kg
Povidone K29-32[a]	25 g	37.5 g	50 g	62.5 g	75 g	87.5 g	100 g	—
2-Propanol	275 ml	262.5 ml	250 ml	237.5 ml	225 ml	212.5 ml	300 ml	—
Gelatin	—	—	—	—	—	—	—	30 g

[a] General Aniline and Film Corp., New York, NY 10020.

of the granules was used. A weight (w) of 60 g of granules of the 14/20-mesh fraction was charged into the cylinder of the described ball mill. One hundred steel balls, 0.53 cm in diameter, and weighing 0.993 g each, were charged into the cylinder, which was then closed. The ball mill was operated at 120 rpm for a certain time (t min). The material was then sieved, and the amount retained on a 20-mesh screen (w_a g) was determined.

Sieve analysis was performed on the minus 20-mesh fraction (40, 60, 80, and 100 mesh). The weight mean diameter, d_b, of the material passing the 20-mesh screen was determined (by methods described later). Ball milling of a 20/40-mesh cut was also carried out; in this case, a 40-mesh sieve was used to determine w_a.

RESULTS AND DISCUSSION

Kick's law (5, 6) states that the energy, E, expended in milling a material of particle size d' cm into a material of particle size d'' cm is given by:

$$E = -C \ln(d''/d') \qquad \text{(Eq. 1)}$$

where C is a constant particular to the milled material. In the present study, milling was performed on either a 14/20-mesh granule, *i.e.*, a granule with the mean diameter $d_a = 1135$ μm, or a 20/40-mesh granule, *i.e.*, a granule with mean diameter $d_a = 638$ μm. Ball milling produced a fine powder, *i.e.*, a powder passing a 20-mesh screen (when 14/20-mesh granules were milled) or a 40-mesh screen (when 20/40-mesh granules were milled), with a mean weight diameter, d_b, that changed little with time.

If w g of material is milled for t min and it then is determined that w_a g of material of original size remains and that $w_b = w - w_a$ g of fine material has been produced, then:

$$d_a = d' \qquad \text{(Eq. 2)}$$

and:

$$d'' = \frac{(w_a d_a + w_b d_b)}{w} \qquad \text{(Eq. 3)}$$

The energy input is proportional to t, *i.e.*:

$$E = qt \qquad \text{(Eq. 4)}$$

Combining Eqs. 1–4 then gives:

$$\ln\left\{\frac{w_a}{w} + \frac{d_b w_b}{d_a w}\right\} = -kt \qquad \text{(Eq. 5)}$$

where:

$$k = q/C \qquad \text{(Eq. 6)}$$

with k in units of minutes^{-1}. To evaluate k, it is necessary to determine w_a and d_b. Therefore, it is necessary to perform a sieve analysis of the material passing through the 20- (or 40-) mesh sieve at each time point, t.

Table II—Sieve Analysis Data for Formula II (14/20 Mesh) Milled for 2 min

Mesh	Sieve Opening, μm	Amount on Mesh, g	Fraction
20	840	64	0.54
40	420	34	
60	250	3	
80	177	1	0.46
100	149	1	
Pan	0	16	

The data treatment of the results from the four granulations where both 14/20- and 20/40-mesh sieve fractions were studied are listed in Tables II–IV. The sieve analysis data will typically appear as shown in Table II. The parameters of importance are w_a/w, which is the fraction remaining on a 20-mesh screen when a 14/20-mesh granulation is milled (and a 40-mesh sieve when a 20/40-mesh granulation is milled). The value of w_b/w is obtained directly as $1 - (w_a/w)$. The mean particle diameter, d_b, of the fine fraction is obtained by converting the data as they appear in Table II to a fraction smaller than the mean sieve diameter (M μm) and then converting these latter values to normalized standard deviates (τ). A complete set of data of this type corresponding to Formula II, 14/20 mesh, is given in Table III. The columns headed "2 min" in Table III correspond to the data in Table II.

The appropriate fit of the data is log normal (5, 6). Data from other types of fitting are not shown, but it is apparent from the graphical presentation of the results in Fig. 1, as well as from the good correlation coefficients (r^2) shown in Table III, that the data adhere to:

$$\tau = a + b \ln M \qquad \text{(Eq. 7)}$$

where a and b are intercepts and slopes of the log-normal plot. The mean diameter, d_b, is then obtained by equating τ with zero, *i.e.*:

$$\ln d_b = -a/b \qquad \text{(Eq. 8)}$$

The least-squares fit data by this treatment are shown in Table III. Since d_b is (fairly) invariant with time, the data were pooled and the least-squares parameters were obtained for each formula. For Formula II, 14/20 mesh, these parameters are:

$$\tau = 4.0462 - 0.6822 \ln M \qquad r^2 = 0.91 \qquad \text{(Eq. 9)}$$

$$d_b = 376 \pm 6\ \mu\text{m} \qquad \text{(Eq. 10)}$$

The 95% confidence limits for d_b are narrow because the interpolation is close to the average τ value and this proximity is statistically optimum. Table IV lists the d_b values obtained from the four granulations (II, IV, VI, and VIII). In practice, it is time consuming to perform a complete sieve analysis and large sample sizes are required. In actuality, a complete sieve analysis is not necessary. If only w_a is recorded, one can, by iterative procedures, obtain d_b. An example of this approach is shown in Fig. 2, where the data from Formula I are treated according to Eq. 5 by inserting consecutive values of d_b. Underestimation of d_b gives curvature toward the abscissa, and overestimation gives curvature away from the abscissa. Values of k obtained this way or by inserting experimental d_b values in Eq. 5 are shown in Table V.

Ideally, the plots adhering to Eq. 5 should have intercepts of zero and correlation coefficients of $r^2 = 1$. Table V shows the correlation coefficients significant at the 95 or 99% confidence level and intercepts that do not differ significantly ($\alpha = 0.05$) from zero.

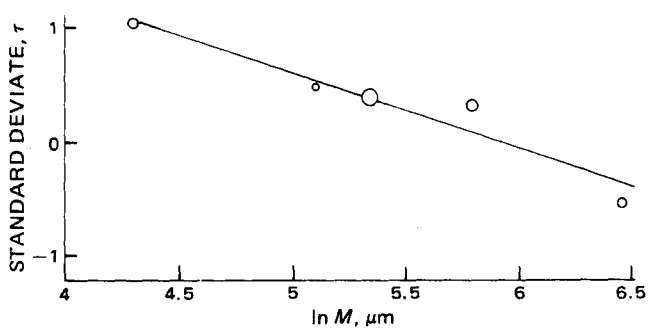

Figure 1—*Log-normal plot of fine fraction from Formula II (14/20 mesh). Each point represents the average of four time points, and the size of the circle represents the range of* t *values it represents.*

Table III—Sieve Analysis as a Function of Time for Formula II [a] (14/20 Mesh)

Mesh Mean, M, μm	Cumulative Fraction below M				τ-Values from Cumulative Fraction Smaller than M			
	2 min	4 min	6 min	8 min	2 min	4 min	6 min	8 min
630	0.69	0.70	0.71	0.72	−0.50	−0.52	−0.56	−0.57
335	0.35	0.37	0.39	0.40	0.37	0.33	0.28	0.38
213	0.32	0.33	0.34	0.35	0.47	0.44	0.40	0.35
163	0.30	0.31	0.32	0.32	0.52	0.50	0.48	0.48
	0.14	0.15	0.15	0.15	1.06	1.05	1.03	1.04
				r^2	0.91	0.92	0.93	0.89
				a	4.03	4.04	4.07	4.04
				b	−0.67	−0.68	−0.69	−0.68
				$\ln d_b$	5.99	5.95	5.89	5.91
				d_b, μm	398	382	360	368

[a] Formula II was studied for 8 min only since only 4% was left on the 20-mesh screen after 8 min.

Table IV—Particle-Size Statistics for Four Granulations

Formula	Povidone, g	Original Mesh Fraction	r^2	a	b	d_b, μm[a]	d_a, μm
II	37.5	14/20	0.91	4.05	−0.68	376 ± 6	1135
IV	62.5	14/20	0.89	4.42	−0.71	507 ± 10	1135
VI	87.5	14/20	0.92	5.03	−0.80	527 ± 7	1135
VIII	Gelatin	14/20	0.95	5.84	−0.93	524 ± 10	1135
II	37.5	20/40	0.95	5.28	−1.00	198 ± 5	630
IV	62.5	20/40	0.92	5.33	−0.98	226 ± 10	630
VI	87.5	20/40	0.90	5.62	−1.07	247 ± 4	630
VIII	Gelatin	20/40	0.91	6.07	−1.08	271 ± 8	630

[a] The 95% confidence limits on the interpolated value for = 0 (see text).

Table V—Attrition Constants and Harwood–Pilpel Crushing Strengths

Formula	Povidone, g	d_a[a], μm	d_b[b], μm	k, min^{-1}	Intercept	r^2	ln Q, g/mg
I	25	1135	100 (250)	(0.195)	−0.002	0.994	4.98
II	37.5	1135	376	0.150	−0.008	0.942	4.90
III	50	1135	470 (450)	(0.087)	0.020	0.997	5.48
IV	62.5	1135	507	0.061	−0.040	0.974	5.50
V	75	1135	540 (500)	(0.060)	0.030	0.990	5.69
VI	87.5	1135	527	0.046	−0.021	0.981	5.72
VII	100	1135	530 (550)	(0.046)	−0.012	0.997	5.94
VIII	Gelatin	1135	524	0.19	−0.007	0.955	6.40
II	37.5	630	198	0.132	−0.069	0.999	
IV	62.5	630	226	0.073	0.048	0.993	
VI	87.5	630	247	0.042	0.031	0.991	
VIII	Gelatin	630	27	0.014	−0.020	0.996	

[a] The 1135 implies that 14/20 material was used; 630 implies that 20/40-mesh material was used. [b] Figures in parentheses imply that the values were obtained through iteration or from iterated values.

The data in Table V are shown graphically in Fig. 3, where Harwood–Pilpel crushing strengths, Q (in grams per milligram), are plotted *versus* attrition constants (in minutes^{-1}) on a ln–ln scale. The data give a least-squares relation of:

$$\ln Q = -0.652 \ln k + 3.808 \qquad \text{(Eq. 11)}$$

with a correlation coefficient of $r^2 = 0.96$, significant for $N = 8$ at the 95% level. Therefore, there is a correlation between Harwood–Pilpel crushing strength and attrition coefficients for at least two granulations, and it is presumably feasible to substitute one for the other as a granule "hardness" test. The advantage of the attrition constant is its lower variance (coefficient of variation of 1–5%) vis-a-vis that of the crushing strength (coefficient of variation of 50–80%).

The practical utility of this correlation is that, if a laboratory has a ball mill, k of a particular mesh is a measure of the crushing strength of the

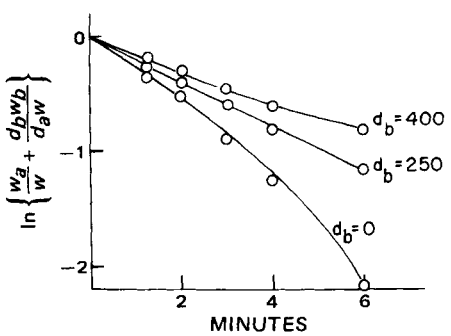

Figure 2—*Estimation of* d_b *via iterative procedure and Eq. 5. Key: lower curve, estimate of* $d_b = 0$*; upper curve, estimate of* $d_b = 400$ *μm; and middle (linear) curve, estimate of* $d_b = 250$ *μm.*

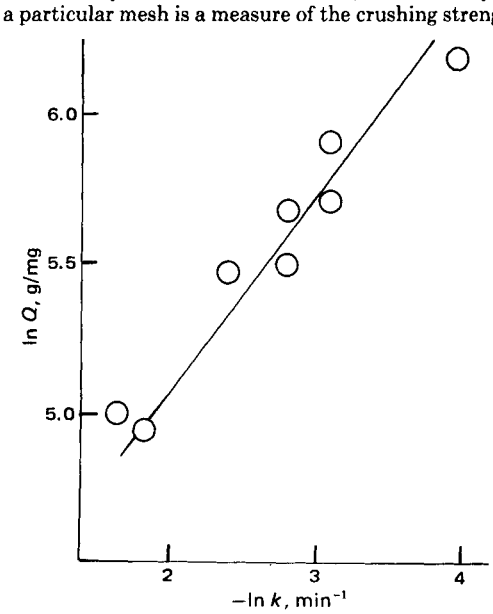

Figure 3—*Correlation between −ln* k *and ln* Q.

granulation and can be used as a process parameter to evaluate batch-to-batch as well as formula-to-formula variation. If this procedure is followed, it is worthwhile at one time (only) to correlate the k values to the Q values for a particular sieve fraction of a particular granulation using the equipment (ball mill, rotations per minute, and steel spheres) desired for the testing.

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General Treatment of Competitive Binding of Small Molecules to Macromolecules as Applied to Dynamic Dialysis: Theoretical Analysis

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Abstract □ A mathematical analysis of the dynamic dialysis process is presented, demonstrating how the process can be applied generally to study competitive and noncompetitive binding between small molecules and macromolecules. A law of mass action model for competitive binding with independent sites and classes with equivalent sites (CIE) is considered as a specific case without loss of generality. The escape profiles of two compounds are calculated to illustrate the effect of an increasing degree of binding competition. Noisy data are generated using the CIE model to test the presented method of estimating competitive binding parameters. The parameters estimated by the nonlinear regression technique came close to the true values, considering the degree of noise added to the exact dialysis data. A transformation approach is presented, enabling initial estimates of the binding parameters in the CIE model to be determined by multiple linear regression, thereby eliminating the main problem in the nonlinear estimation. The presented method of analysis is extended to strongly bound compounds, which also bind significantly to the dialysis membrane.

Keyphrases □ Dynamic dialysis—mathematical analysis, competitive binding of small molecules to macromolecules studied □ Models, mathematical—law of mass action for competitive binding with independent sites and classes with equivalent sites □ Binding, competitive—small molecules to macromolecules, mathematical analysis of dynamic dialysis process

Dynamic dialysis has proven valuable for characterizing interactions of small molecules with macromolecules such as drugs and proteins (1–11). Experimentally, it appears to be the simplest and most convenient method available for determining a complete binding profile (3), and its accuracy seems to be as good as equilibrium dialysis and ultrafiltration methods (3). The main disadvantages of dynamic dialysis are the inaccuracy introduced by the classical data treatment (9, 12), which requires differentiation of discrete data (3), and its limitation to molecules that do not bind or adsorb significantly to the dialysis membrane. However, both disadvantages recently were eliminated by a new approach in the data treatment (12).

This paper presents a mathematical analysis that enables the dynamic dialysis process to be extended to the study of competitive binding between small molecules and macromolecules considering any mathematical model for such interaction.

THEORY

To illustrate the general approach, it is appropriate to consider a law of mass action model with competitive binding, independent sites, and equivalence between sites in the binding classes having multiple sites:

$$\bar{\nu}_i = \sum_{j=1}^{N} n_j k_{ij} c_i \left[1 + \sum_{m=1}^{M} k_{mj} c_m\right]^{-1} \qquad \begin{matrix} i = 1, 2, \ldots, M \\ k_{ij} \geq 0 \end{matrix} \qquad \text{(Eq. 1)}$$

This model will be denoted the general CIE model, where $\bar{\nu}_i$ is the number of moles of the ith small molecules ("ligand") bound per mole of macromolecule, n_j is the number of equivalent binding sites in the jth class of sites, k_{ij} is the association constant for the ith compound's binding to the jth binding class, c_i is the free concentration of the ith compound, N is the number of binding classes, and M is the number of compounds competing in their binding to the macromolecule. Most frequently, $M = 2$. If $M = 1$, the CIE model reduces to the general (IE) model, dealing with the binding of one compound (12).

Cases where the compounds compete in their binding to certain, but not all, of the binding classes also are considered in the CIE model by allowing the association constants to take zero values (*i.e.*, $k_{ij} \geq 0$). For example, Compound 1 may bind to two classes of sites, and Compound 2 may bind to two classes. If the two compounds only compete in their binding for one class (*e.g.*, class two for Compound 1 is the same as class one for Compound 2), this situation is described by $k_{11} > 0$, $k_{12} > 0$, $k_{13} = 0$, $k_{21} = 0$, $k_{22} > 0$, and $k_{23} > 0$. Thus, the actual number of classes is $N = 3$.

According to $\bar{\nu}_i = ([c_i] - c_i)/P$, where $[c_i]$ is the total (free plus bound) molar concentration of the ith compound and P is the total molar concentration of macromolecule, Eq. 1 can be written:

$$[c_i] = c_i \left\{1 + P \sum_{j=1}^{N} n_j k_{ij} \left[1 + \sum_{m=1}^{M} k_{mj} c_m\right]^{-1}\right\} \qquad \begin{matrix} i = 1, 2, \ldots, M \\ k_{ij} \geq 0 \end{matrix} \qquad \text{(Eq. 2)}$$

The dynamic process is characterized by the following relationship:

$$d[c_i]/dt = -K_i c_i \qquad \text{(Eq. 3)}$$

where K_i is the dialysis rate constant for the ith compound. Equations 2 and 3 define the kinetics of dialysis. However, the use of these equations in their present form requires differentiation of discrete data, which introduces substantial errors in the determination of the binding parameters (9, 12).

To avoid such errors, it is necessary to eliminate the variables, c_i, that cannot be measured directly by using a technique similar to that pre-

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