derived from those physical-chemical properties responsible for the modification involved and for the interaction between the dosage form and the physiological surroundings where drug release and drug absorption takes place. The best way to avoid any possible discrepancy between real behavior and that inferred from the ANOVA is to carefully choose the interval and the number of levels necessary for the study. The cost of the *in vivo* experiment necessarily limits the number of levels studied.

The dissolution accumulative curves obtained are shown in Fig. 3, while the mean values of the chosen parameters, D_{30} and D_{180}, are listed in Table V. A quadratic response for both parameters, with respect to the fat content, can be inferred from the one-way ANOVAs shown in Tables VI and VII. The polynomials, after least-squares adjustment, were:

$$D_{180} = 300.090 + 70.924\,x - 2.593\,x^2 \qquad \text{(Eq. 7)}$$

$$D_{30} = 1549.010 - 112.343\,x + 2.053\,x^2 \qquad \text{(Eq. 8)}$$

They are shown in Fig. 4. It is worth noting that this excipient exercises the same influence *in vitro* as it does *in vivo*.

Bioequivalence studies carried out on formulations whose composition is defined by an appropriate experimental design afford the opportunity of studying the effect of the different excipients on bioavailability; that is, the effect of these excipients on the quantity of drug absorbed and on the rate of absorption. The effect the excipients have on interindividual variability must also be considered. As has been pointed out previously (16, 17), it is not sufficient just to accept the null hypothesis for the parameters employed as an indication of bioequivalence between two formulations; the variability must also be similar.

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Quantification of the Effect of Excipients on Bioavailability by Means of Response Surfaces II: Amoxicillin in Fat–Silica Matrix

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Received April 11, 1980, from the *Departamento de Farmacia Galénica, Facultad de Farmacia, Universidad de Santiago de Compostela, Spain.* Accepted for publication November 17, 1981.

Abstract □ This report studies the bioavailability of amoxicillin in different fat–silica matrixes. A urinary excretion study was carried out on four formulations containing fat and silica excipients. The formulations were administered to 24 healthy volunteers according to a Latin-square design. The following percent proportions of fat–silica were used: 15:3.75, 15:7.50, 30:3.75, and 30:7.50. The urinary excretion curves were characterized using the quantity of unchanged drug excreted between 0–2 and 0–12 hr postadministration, respectively as parameters. The ANOVA results showed that both excipients had an additive effect on the quantity of drug excreted between 0 and 2 hr, whereas the effect on the quantity of drug excreted between 0 and 12 hr was also one of interaction between both excipients. Quantification of the ANOVA results in terms of excipient content was conducted by means of the adequate linear functions. At the same time, a dissolution study was carried out using the quantity of drug dissolved in 30 and 180 min as parameters. The behavior was similar to that encountered for the *in vivo* parameters.

Keyphrases □ Amoxicillin—effect of excipients on bioavailability by means of response surfaces, fat–silica matrix □ Bioavailability—effect of excipients by means of response surfaces, amoxicillin in fat–silica matrix □ Excipients—effect on bioavailability by means of response surfaces, amoxicillin in fat–silica matrix

The aim of the present study is to determine the effect which the combination of two excipients, a synthetic fat[1] and a silica colloid[2], has on the bioavailability of amoxicillin tablets.

EXPERIMENTAL

Assayed Formulations—Four formulations of amoxicillin trihydrate[3] tablets were manufacturerd and studied. Formulations, D, E, F, and G, contained 375 mg of anhydrous amoxicillin, and their percent composition is shown in Table I. Hardness, in each case, was 5 kg on the hardness tester scale[4].

Clinical Protocol—The urinary excretion of unchanged drug was studied in 24 healthy volunteers whose ages ranged from 20 to 30 years and who showed no evidence of renal insufficiency. The subjects were randomly divided into four equal groups. A Latin-square 4 × 4 design with 6 replicates was used, and the washing period was 5 days. Immediately before a standard breakfast, fasted subjects were given two tablets equivalent to 750 mg of anhydrous amoxicillin. Urine samples were collected at 1, 2, 3, 4, 5, 6, 8, 10, and 12 hr postadministration.

Pharmacokinetic Analysis—Characterization of the urinary excretion curves was achieved by means of the parameters mentioned in the previous report (1).

Dissolution Rate Studies—The apparatus and methodology used were both described in the previous report (1).

Statistical Analysis—Heterogeneous variances for the treatments

[1] Precirol, Gattefossè.

[2] Aerosil, Degussa.

[3] Antibióticos S.A. lot A30H-106, potency 859 μg/mg.

[4] Monsanto hardness tester.

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Table I—Percent Composition of Formulations

Formulation	Amoxicillin Trihydrate, %	Fat, %	Silica, %	Talc, %
D	77.25	15	3.75	5
E	72.50	15	7.50	5
F	62.25	30	3.75	5
G	57.50	30	7.50	5

Table II—Mean Values and Variances [a] for the Excretion Parameters

Formulation	E_2	E_{12}
D	36.2 (763.3)	237.6 (11,322.6)
E	84.2 (4,407.8)	352.9 (16,708.0)
F	16.9 (113.7)	112.8 (3,366.3)
G	52.5 (1,982.4)	353.4 (7,306.9)

[a] Variances in parentheses.

were observed and exposed by means of Barlett's test (2). Once again, logarithmic transformation of data failed to stabilize the variances; therefore, Scheffé's test, modified by Brown and Forsythe (3), was again employed.

The study was carried out on four formulations combining two equally spaced levels of each of the excipients. Therefore, the subdivision of the sum of squares for the term treatments of the ANOVA, to obtain the response surfaces, was achieved by means of the following polynomials (Z):

Treatment	Z_1	Z_2	Z_3
T_1	−1	−1	1
T_2	1	−1	−1
T_3	−1	1	−1
T_4	1	1	1
Component	Fat mean response	Silica mean response	Interaction

These polynomials subdivide the sum of the squares in mean response for each excipient and interaction between the two excipients (4).

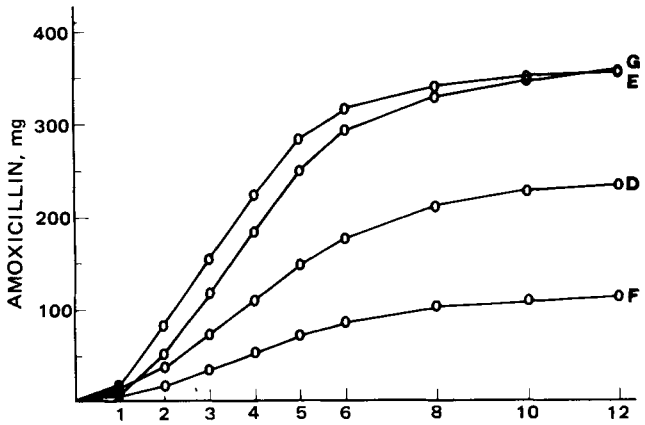

Figure 1—*Cumulative curves for urinary excretion of unchanged amoxicillin.*

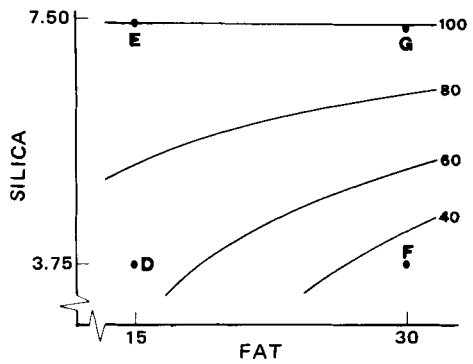

Figure 2—*Contour curves for* E_{12} *as function of fat and silica contents.*

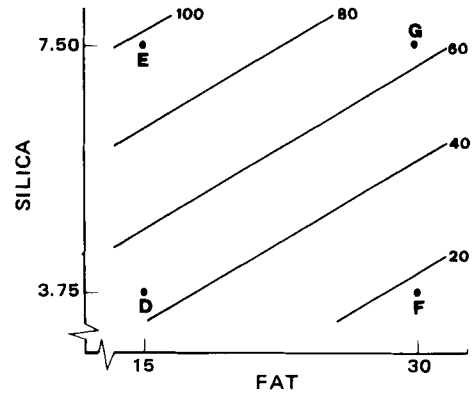

Figure 3—*Contour curves for* E_2 *as function of fat and silica contents.*

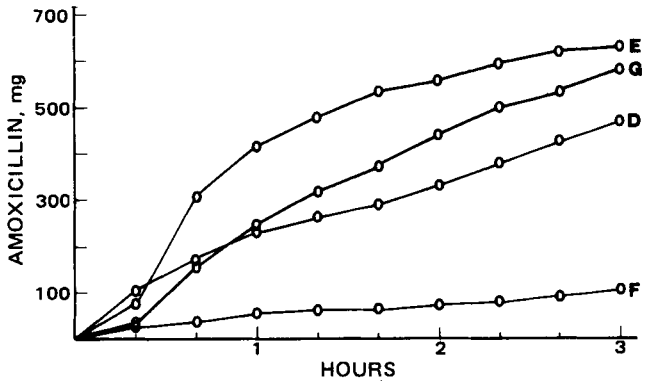

Figure 4—*Cumulative dissolution curves.*

Having inferred the factors that affect absorption, and the existence or nonexistence of interaction, the corresponding polynomial functions were then adjusted to the mean experimental values by means of the least-squares method. The results are shown graphically by means of the contour lines generated by these equations.

RESULTS AND DISCUSSION

The mean urine excretion curves are depicted in Fig. 1, whereas the mean values of the parameters used for their characterization, E_2 and E_{12}, and the respective variances, are shown in Table II. The ANOVA results appear in Tables III and IV. As mentioned previously (1), two parts must be distinguished, namely, the contrasts between pairs of formulations and the orthogonal contrasts used to monitor the effect of the excipients on bioavailability. The latter contrast shows that both excipients have a significant effect on the two parameters. With respect to the E_{12} parameter, significant interaction between both excipients is also observed. According to this, the following functions indicate the quantitative correlation between the bioavailability parameters and the fat (x_1) and (x_2) content, expressed as percent, for those formulations

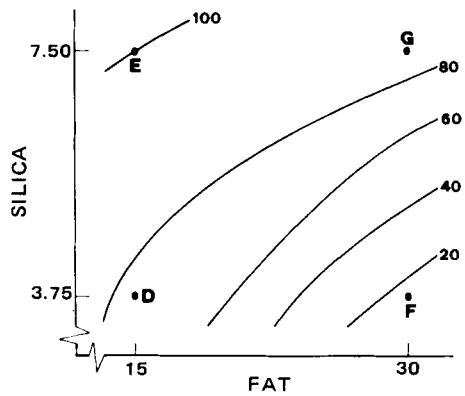

Figure 5—*Contour curves for* D_{180} *as function of fat and silica contents.*

Table III—Results of ANOVA for E_{12} [a]

i	H_i	$V(H)$	$g-1$	f	F	A	Results
1	−124.27	1612.66	3	73	2.72	114.71	Significant fat effect
2	355.85	1612.66	3	73	2.72	114.71	Significant silica effect
3	125.23	1612.66	3	73	2.72	114.71	Significant fat–silica interaction
4	−124.75	612.04	3	73	2.72	74.83	Significant contrast D *versus* F
5	115.31	1167.94	3	73	2.72	103.28	Significant contrast D *versus* E
6	115.79	776.23	3	73	2.72	84.28	Significant contrast D *versus* G
7	240.06	836.43	3	73	2.72	87.48	Significant contrast F *versus* E
8	240.98	444.71	3	73	2.72	63.79	Significant contrast F *versus* G
9	−0.48	1000.62	3	73	2.72	95.69	No significant contrast E *versus* G

[a] $\alpha = 0.05$. The minimum value for rejecting the null hypothesis is $A = \sqrt{(g-1)\,FV\,(H_i)}$ (Ref. 3).

Table IV—Results of ANOVA for E_2 [a]

i	H_i	$V(H)$	$g-1$	f	F	A	Results
1	−50.80	302.80	3	73	2.72	49.71	Significant fat effect
2	83.80	302.80	3	73	2.72	49.71	Significant silica effect
3	−12.46	302.80	3	73	2.72	49.71	No significant fat–silica interaction
4	−19.21	36.54	3	73	2.72	18.28	Significant contrast D *versus* F
5	48.03	215.46	3	73	2.72	44.40	Significant contrast D *versus* E
6	16.36	114.40	3	73	2.72	32.35	No significant contrast D *versus* G
7	67.24	188.40	3	73	2.72	41.52	Significant contrast F *versus* E
8	35.37	87.44	3	73	2.72	28.27	Significant contrast F *versus* G
9	−31.67	266.26	3	73	2.72	43.36	No significant contrast E *versus* G

[a] $\alpha = 0.05$ (see Table III).

Table V—Mean Values for the Chosen Dissolution Parameters

Formulation	D_{30}	D_{180}
D	141.3	470.0
E	204.8	634.0
F	49.3	106.3
G	135.4	580.0

Table VI—Results of ANOVA for D_{180}

Source of Variation	Sum of Squares	Degrees of Freedom	F	α
Treatments	462,925.01	3	35.23	<0.01
Fat	169,218.75	1	38.63	<0.01
Silica	242,013.44	1	55.25	<0.01
Fat and Silica	51,692.81	1	11.80	<0.01
Residual	35,044.37	8		
Total	497,969.37	11		

Table VII—Results of ANOVA for D_{30}

Source of Variation	Sum of Squares	Degrees of Freedom	F	α
Treatments	85,891.83	3	63.26	<0.01
Fat	70,878.76	1	156.61	<0.01
Silica	14,195.50	1	31.37	<0.01
Fat and Silica	817.58	1	1.81	—
Residual	3,620.63	8		
Total	89,512.46	11		

whose composition lie within the limits of the experiment:

$$E_{12} = 372.17 - 16.66\,x_1 - 2.63\,x_2 + 2.22\,x_1x_2 \qquad \text{(Eq. 1)}$$

$$E_2 = 22.90 - 1.70\,x_1 + 11.15\,x_2 \qquad \text{(Eq. 2)}$$

The response surfaces obtained are shown in Figs. 2 and 3. A value of 100% is assigned to Formulation E, to which the other formulations are referred. Observation of Fig. 2 leads to the conclusion that, in order to obtain acceptable physiological availabilities within the limits of the experiment, a silica content close to 7.5% should be used, no matter what the fat content may be; because at this level, the silica practically cancels out the effect of the fat on the E_{12} parameter. For this reason, Formulations E and G present equivalent absorption. Nevertheless, with low silica concentration (3.75%), the fat content produces a marked effect as can be seen by the differences observed between Formulations D and F. The response surface obtained for the E_2 parameter plane surface in this case, due to the nonexistence of interaction between excipients, is shown in Fig. 3. The observed response corresponds to the sum of the effects produced by both excipients: negative with regard to the fat content and positive in the case of silica. For this reason, Formulation E, with low content in fat and high content in silica, gives the highest value for the E_2 parameter and also shows significant differences with regard to the other formulations. It is also seen that those formulations characterized by incomplete absorption show higher variation coefficients, as pointed out previously (5).

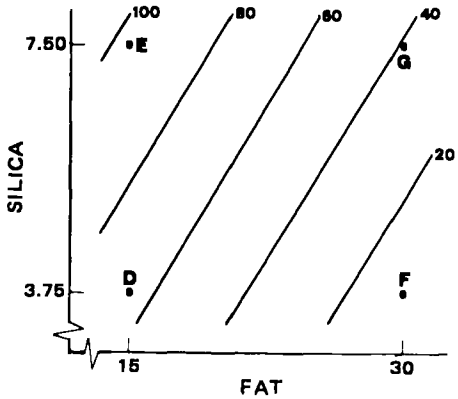

Figure 6—*Contour curves for* D_{30} *as function of fat and silica contents.*

With respect to the dissolution tests, the mean accumulative curves appear in Fig. 4, while the mean values of the chosen parameters are shown in Table V. The respective ANOVAs are shown in Tables VI and VII. It can inferred from Table VII that the two excipients have a significant effect on both parameters, whereas in the case of the D_{180} parameter, there is also significant interaction between both excipients. According to this, the following functions indicate the quantitative correlation between the dissolution parameters and the fat (x_1) and silica (x_2) content, expressed as a percent, for those formulations whose composition lie within the bounds of this study:

$$D_{180} = 964.39 - 42.06\,x_1 - 29.16\,x_2 + 4.66\,x_1x_2 \qquad \text{(Eq. 3)}$$

$$D_{30} = 267.20 - 10.25\,x_1 + 18.34\,x_2 \qquad \text{(Eq. 4)}$$

Commentaries similar to those made about the *in vivo* response may also be made about the response surfaces obtained *in vitro* which are depicted in Figs. 5 and 6. Nevertheless, with the conditions used in the dissolution studies, the order relationship *in vivo* (G, E, D, F, for E_{12} and E, G, D, F, for E_2) is not the same as *in vitro* (E, G, D, F for D_{180} and E, D, G, F for D_{30}).

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Quantification of the Effect of Excipients on Bioavailability by Means of Response Surfaces III: *In Vivo–In Vitro* Correlations

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Received April 11, 1980, from the *Departamento de Farmacia Galénica, Facultad de Farmacia, Universidad de Santiago de Compostela, Spain.* Accepted for publication November 17, 1981.

Abstract □ This study compares one of the previously studied formulations with commercial amoxicillin capsules. The results indicate that the percentage of the dose absorbed is similar in both formulations; nevertheless, the amoxicillin capsules present a higher absorption rate. The *in vivo–in vitro* correlations in terms of response surfaces, and the general correlation among all the formulations studied in the three articles of this series is discussed. The quantity of drug excreted in 2 hr and the quantity of drug dissolved in 30 min presents a correlation coefficient $r = 0.9458$ ($p < 0.01$) and the quantity of amoxicillin excreted in 12 hr and the quantity dissolved in 180 min presents a correlation coefficient $r = 0.9761$ ($p < 0.01$).

Keyphrases □ Amoxicillin—effect of excipients on bioavailability by means of response surfaces, *in vivo–in vitro* correlations □ Bioavailability—effect of excipients by means of response surfaces, amoxicillin, *in vivo–in vitro* correlations □ Excipients—effect on bioavailability by means of response surfaces, amoxicillin, *in vivo–in vitro* correlations

The comparison between Formulation E (1), previously studied, and a commercial amoxicillin capsule (Formulation S) was carried out. This study has a double purpose: first, to determine whether the absorption of the drug in Formulation E (which showed good absorption in previous studies) is equivalent to the absorption shown by the conventional formulations; second, to determine the degree of the individual variation for both types of formulations.

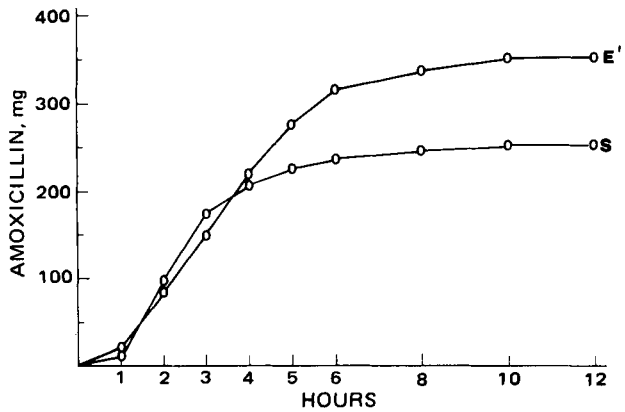

Figure 1—*Mean cumulative curves for urinary excretion of unchanged amoxicillin.*

EXPERIMENTAL

Assayed Formulations—A comparison is made between Formulation E, whose composition has been described (1) and commercial capsules[1] containing 500 mg of anhydrous amoxicillin. To differentiate the results of the present study from those obtained previously (1), Formulation E is termed E′ in the present report.

Clinical Protocol—Urinary excretion of unchanged drug was studied in 12 healthy volunteers of both sexes, whose ages ranged from 20 to 30 years and who showed no evidence of renal insufficiency. Subjects were divided at random into two equal groups of six. A Latin-square 2 × 2 design with 6 replicates was used, and the washing period was 5 days. The conditions of administration and sample times are the same as those described in the previous two reports (1, 2).

Pharmacokinetic Analysis—The parameters employed to characterize the excretion curves obtained are the same as those described previously (1, 2), E_2 and E_{12}.

Dissolution Rate Studies—The apparatus and methodology used were both described in Part I (2).

Statistical Analysis—Cochran's test (3) confirmed the existence of heterogeneous variances for the treatments. Therefore, Scheffé's test modified by Brown and Forsythe (4) was employed (even though the logarithmic transformation of data stabilized the variances) in order to maintain homogeneity in the statistical treatment of data.

RESULTS AND DISCUSSION

A commercial formulation of 500 mg was chosen because the urinary excretion curves obtained for Formulation E in the previous study indicated the possibility of obtaining peaks similar to those obtained with 500-mg doses in conventional formulations. This approach, together with the comparison of the parameters E_2 and E_{12}, permits the possibility of determining whether or not there is a prolongation in the release–absorption process with respect to Formulation E′. This would explain the large individual variations observed in previous studies.

Figures 1 and 2 show the excretion and dissolution mean cumulative curves for both formulations. With respect to the E_2 parameter, the mean values and variances (in parentheses) obtained for Formulations E′ and S were 85.3 (2824.2) and 98.1 (2188.9), respectively. The corresponding figures for the E_{12} parameter were 354.4 (9003.6) and 253.6 (1895.9). The dissolution parameter D_{30} yielded mean values of 204.8 and 404.0 for Formulations E′ and S, respectively, whereas the D_{180} parameter yielded mean values of 634.0 and 471.5 for the respective formulations. The ANOVA results, using the method of Brown and Forsythe (4), show that both formulations are equivalent with regards to the E_2 parameter ($F_{1,22} = 0.392$) but differ significantly (0.01) with regards to the E_{12} parameter ($F_{1,22} = 11.190$). The quantity of intact drug excreted in urine was ~50% of the administered dose in the case of each formulation, which has been

[1] Clamoxil, lot 2L26, F. Bonet.

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