Full Length Research Paper

In vitro-in vivo correlation of four commercial brands of aspirin tablets marketed in Nigeria

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The study investigated the possibility of developing an *in vitro – in vivo* correlation for four commercial brands of aspirin tablets using USPXXI rotating basket apparatus and urinary excretion profiles from eight human volunteers. Various dissolution and pharmacokinetic parameters were obtained for all the brands. Significant rank order correlations were observed between all the *in vitro* dissolution parameters such as percent dissolved at 30 min, dissolution rate constants (k) and time for 50% dissolution ($DT_{50\%}$) and all the *in vivo* bioavailability parameters such as cumulative amount excreted up to 8 h (E_8), maximum excretion rate $(dE/dt)_{max}$ and time for maximum excretion rate (T_{max}). However, no correlation could be established between the cumulative amount excreted up to 24 h (E_{24}) and any of the *in vitro* dissolution parameters. Moreover, statistical analysis showed no significant inter-subject variation among the subjects that participated in the experiments.

Key words: Aspirin tablets, dissolution rate constant, maximum excretion rate, cumulative amount excreted, *in vitro-in vivo* correlation (IV-IVC).

INTRODUCTION

The formulation and implementation of regulations concerning bioavailability of drugs made considerable attention to be given to correlation of *in vitro* dissolution rate with *in vivo* bioavailability (Emami, 2006). It has been observed that with proper attention to the operating conditions, dissolution test can become a valuable indicator for potential *in-vivo* performance. *In vitro-in vivo* correlation (IV-IVC) is a predictive mathematical treatment describing the relationship between an *in vitro* property of a dosage form (usually the rate or extent of drug release) and a relevant *in vivo* response (e.g. plasma drug concentrations or amount of drug absorbed). Establishing an IV-IVC needs an extensive study of drug release from an oral drug delivery system. The study of drug *in vitro* availability and bioavailability are important part of the IV-IVC procedure for suitable drugs (Xiaohong et al., 2003). In order to develop safe and effective drugs

and validate their formulation, it is important to identify the exact drug pharmacokinetic parameters and biopharmaceutical properties of the dosage forms.

The main objective of establishing IV-IVC is to use dissolution test as a surrogate for *in vivo* bioavailability studies and reduce the need for expensive human studies (Leeson, 1995; Shah and Lesko, 1995). When *in vitro-in vivo* correlations are established on a formulation, dissolution specifications can be used as a means of controlling drug bioavailability and hence a substitute for human bioequivalence studies (Olaniyi et al., 2001). Aspirin belongs to class 2 of Biopharmaceutics Classification System (BCS), because, it has poor water solubility, hence its dissolution rate is the limiting step, which controls the bioavailability parameters of oral aspirin drug products. This criterion makes aspirin a good candidate for IV-IVC study (Amidon et al., 1997; Young et al., 1997). Good correlations between various dissolution and bioavailability parameters of some drugs have been documented (Gadalla et al., 1986; Wood et al., 1990; Hussein and Friedman, 1990; Derendarf et al., 1983;

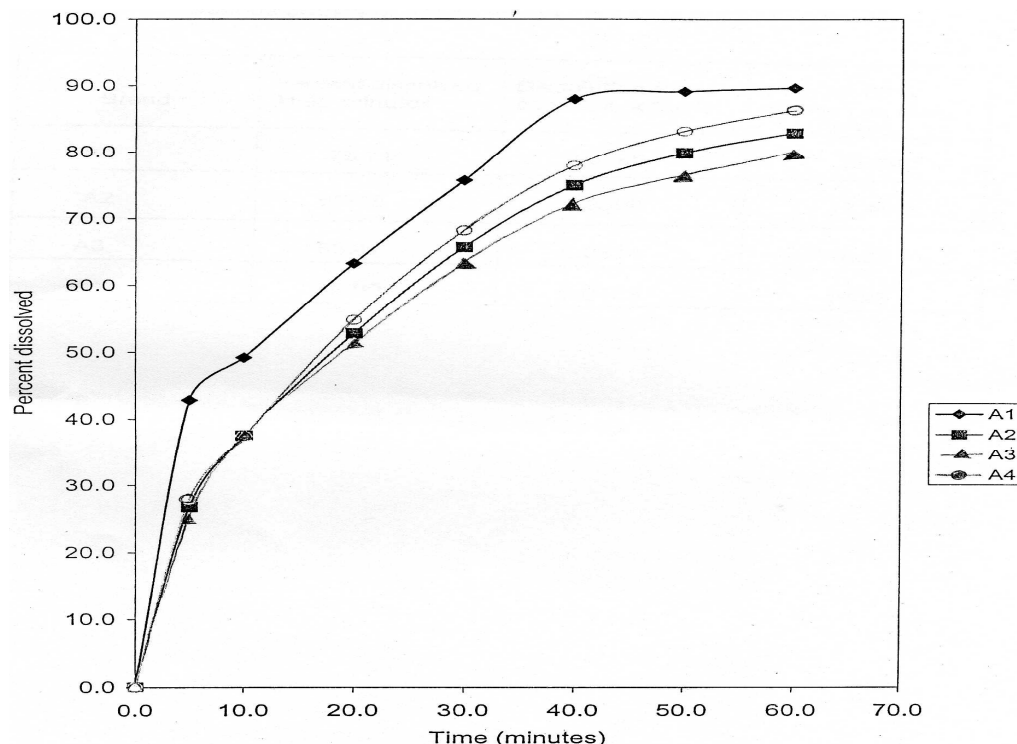*Corresponding author. E-mail: bamigbolae@yahoo.com.

Figure 1. Percent of actual amount of aspirin dissolved as a function of time for the four different brands.

Jung et al., 1997; Abuzarur-aloul et al., 1998). However, in some cases, no good correlation could be obtained as reported by some investigators (Amamar and Khalil, 1993; Gordon et al., 1994; Khan et al., 2009).

The purpose of this study is to investigate the possibility of developing an IV-IVC using four commercial brands of aspirin tablets marketed in Nigeria. There are many categories of IV-IVC. But for the purpose of this study, level C IV-IVC which is a single point relationship between one dissolution parameter and one pharmaco-kinetic parameter will be adopted.

EXPERIMENTAL

The following chemicals were used as procured from the manufacturers without further purification. Aspirin USP fine crystals, salicylic acid USP crystal and sodium salicylate (BDH, England); ferric ammonium sulphate, chloroform and concentrated hydrochloric acid (May and Baker, England). Four commercial brands of aspiring tablets (one soluble brand coded A1 and three plain brands coded A2 to A4) well within their expiry dates were obtained from a retail pharmacy outlet in Jos, Nigeria.

Methods

In vitro dissolution study

The dissolution rate of the aspirin tablets were determined using USPXXI method which employs a rotating basket (Erweka, GmbH, Germany) at 50 rpm in 500 ml of 0.05 M acetate buffer, pH 4.5 ±

0.05 and temperature of 37.0 ± 0.5°C. Samples were withdrawn and filtered at various intervals of 5, 10, 20, 30, 40, 50 and 60 min and analyzed spectrophotometrically at 265 nm using UV-160A spectrophotometer (Spectronic 21, Milton Roy USA). The absorbances were recorded and the amount of aspirin in the samples obtained from the calibration curve of aspirin earlier prepared and the dissolution profiles were obtained for all the brands. The dissolution profiles expressed as percents aspirin dissolved as a function of time for all the brands are shown in Figure 1. Various dissolution parameters such as percent dissolved in 30 min, dissolution rate constants (k) and time for 50% dissolution ($DT_{50\%}$) were obtained for all the brands using standard methods (Shargel and Yu, 1993). These are presented in Table 1.

In vivo bioavailability studies

Selection of subjects

Eight healthy subjects (four male and four female) volunteers that had no history of liver, kidney or gastro-intestinal disease participated in the study after being informed of the purpose and protocol of the study. The participants are aged between 18 to 29 years: Weighing between 50 to 68 kg and having heights between 1.53 to 1.71 m. Subjects did not take any medication, alcohol and other beverages or food that might interfere with the drug, one week prior and throughout the entire study period. Ethical clearance was obtained from the institution's Research Ethics Committee before the start of the experiment. All protocols were carried out according to guidelines stipulated by the committee.

Study design

The study was carried out using a random cross-over design with 7

Table 1. Dissolution parameters obtained from the dissolution tests of the various brands of aspirin tablets.

Brand	Percent dissolved at 30 min	Dissolution rate constant, k (h^{-1})	Time for 50% dissolution, $dt_{50\%}$ (min)
A1	75.71	0.059	11.79
A2	65.75	0.040	17.41
A3	63.47	0.037	18.53
A4	68.90	0.043	16.23

Table 2. Pharmacokinetic parameters obtained from urinary excretion analysis of various brands of aspirin.

Brand	Cumulative amount excreted up to 8 h (E_8)	Cumulative amount excreted up to 24 h (E_{24})	Maximum excretion rate $(dE/dt)max$	Time for maximum excretion rate (T_{max})
A1	173.49	290.59	32.76	2.50
A2	165.15	275.68	29.93	3.38
A3	164.36	247.63	29.64	3.50
A4	169.79	281.10	31.36	3.13

days interval between administrations of each formulation as the washout period. Following an overnight fasts, each subject was asked to void his/her bladder and drink 250 ml of water. In 1 h, the zero-hour urine (blank). Sample was taken as control and 600 mg of aspirin tablets was ingested with another 250 ml of water. No food or liquid other than water was permitted for over 4 h following ingestion of the dose. Cumulative samples were taken at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h.

The volume of the urine samples collected was measured at each collection time. Aliquots of the urine sample were refrigerated immediately and protected from light. Each subject was instructed to drink 250 ml of water after each urine sample collection for the first 3 h and a uniform meal was served after the 4 h sampling.

Urine analysis

The total amount of salicylate excreted in the urine sample was measured using modified colorimetric method (Chio and Onyemelukwe, 1974). 2 ml of concentrated hydrochloric acid was added to 3 ml of urine sample in a screw top pyrex culture tube. After sealing the tubes with caps, they were incubated in a hot air oven at 100°C for 17 h. After cooling to room temperature, 0.5 ml of 5 N hydrochloric acid and 6 ml of chloroform were added. The tubes were shaken mechanically for 10 min and centrifuged for 5 min using a centrifuge (Cliffton Nicket Electron Limited, England). Following centrifugation, 3 ml of the chloroform layer was then accurately transferred to another screw-top culture- tube. A 6 ml of modified Trinder's reagent was added. The tubes were shaken for 10 min and centrifuged again for 5 min. After centrifugation, the absorbance of the aqueous layer was measured at 540 nm using a spectrophorometer (Spectronic 20 UV. Milton Roy, USA) The modified Trinders reagent was used for 100% transmittance adjustment using the calibration curve earlier prepared, the amount of salicylate excreted in the various urine samples were obtained (Joseph, 1984) .

Pharmacokinetic analysis

Using the *in vivo* excretion data obtained, various pharmacokinetic parameters such as cumulative amount excreted up to 8 h (E_8) and 24 h (E_{24}), Maximum excretion rate $(dE/dt)_{max}$ and time for
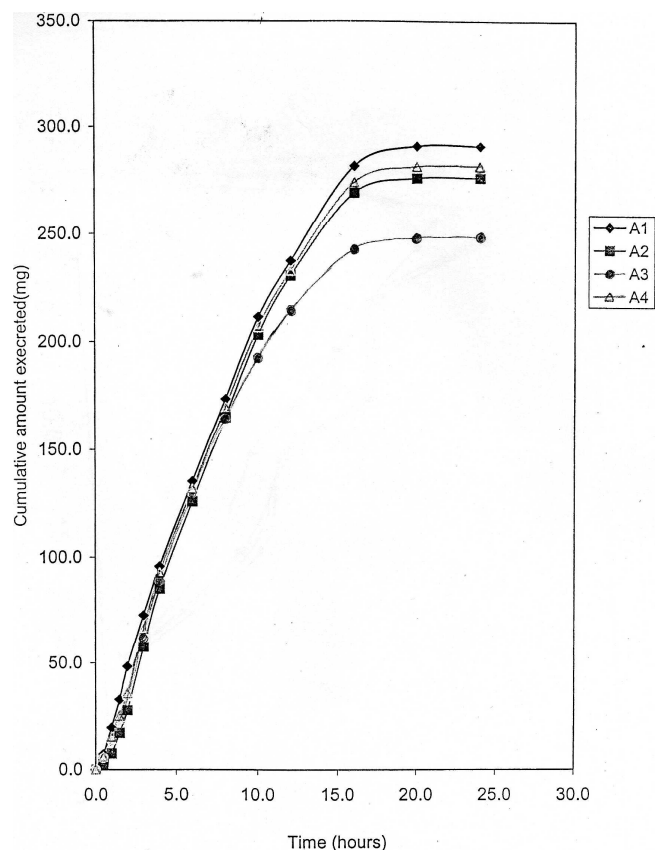
Figure 2. Cumulative amount of salicylate excreted up to 24 h for the different brands of aspirin.

maximum excretion rate T_{max} were calculated using standard methods (Khan et al., 2009) and are presented in Table 2.

The plots of average cumulative amount of salicylate excreted as a function of time and average urinary excretion rate as a function of time are shown in Figures 2 and 3, respectively.
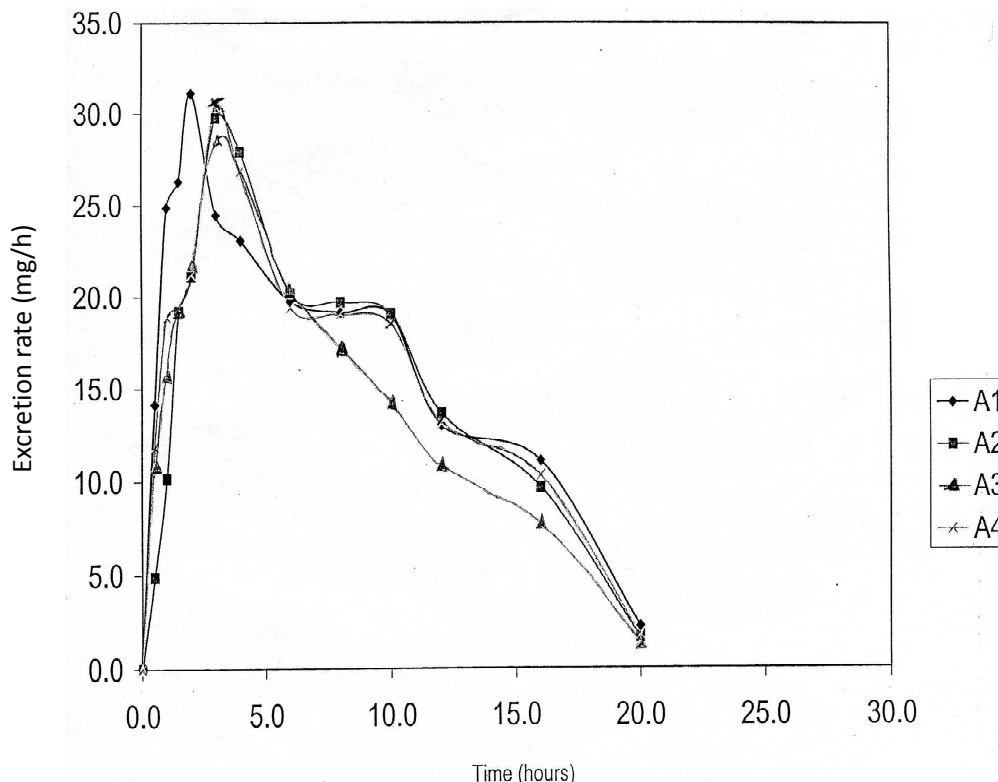
Figure 3. Excretion rate profiles of the four different brands of Aspirin.

RESULTS AND DISCUSSION

Dissolution profile

Figure 1 shows that A1 exhibited the highest dissolution rate while the other three brands (A2 - A4) has similar dissolution rates (p <0.05). Table 1 shows that overall relative ranking of all the brands in terms of the percent dissolved in 30 min and dissolution rate constant (k) followed the order of A1>A4>A2>A3 while the ranking of time for 50% dissolution ($DT_{50\%}$) follows the reverse order (that is, A1<A4<A2<A3). A1 is a soluble brand of aspirin containing calcium carbonate which can provide a reactive medium by changing the pH of the environment adjacent to the drug to alkaline, thus making the acidic drug like aspirin form a water soluble salt, thereby enhancing its rapid dissolution. This possibly accounted for its highest solubility rate and shortest time for dissolution compared with the other plain brands (Serajuddin, 2007). Brands A2, A3 and A4 are all plain aspirin tablets; various factors such as particle size and shape of the aspirin content, type and/or amount of excipients, method of formulation and compression force employed may influence their dissolution rates (Kader and Jalil, 1999; Olaniyi et al., 2001) The analysis of variance (ANOVA) performed on the percent dissolved in 30 min shows no significant difference (P>0.05) among all the brands, they all indicated similar statistical behaviour in their *in vitro* dissolution profiles.

Bioavailability profile

The cumulative salicylate excreted and excretion rate profiles of the soluble aspirin and the three plain aspirin tablets are presented in Figures 2 and 3, respectively while the pharmacokinetic parameters obtained are provided in Table 2. Pharmacokinetic parameters determined were cumulative amount excreted up to 8 h (E_8) and 24 h (E_{24}), maximum excretion rate (dE/dt)max and time for maximum excretion rate (T_{max})

The analysis of variance (ANOVA) performed on the pharmacokinetic parameters shows that all the four brands are bioequivalent in terms of (E_8), (E_{24}) and $(dE/dt)_{max}$, but the T_{max} of the three plain brands are inequivalent to the soluble brand A1 which has the fastest time for maximum excretion rate. Figure 3 shows that curve for A2, A3 and A4 were almost super imposable attesting further to their equivalence. The formulation factors that enhanced the *in vitro* dissolution of the soluble aspirin is possibly responsible for increase in its *in vivo* bioavailability, compared to other plain brands, since its dissolution process is the rate limiting step (Emami, 2006; Gordon et al., 1994).
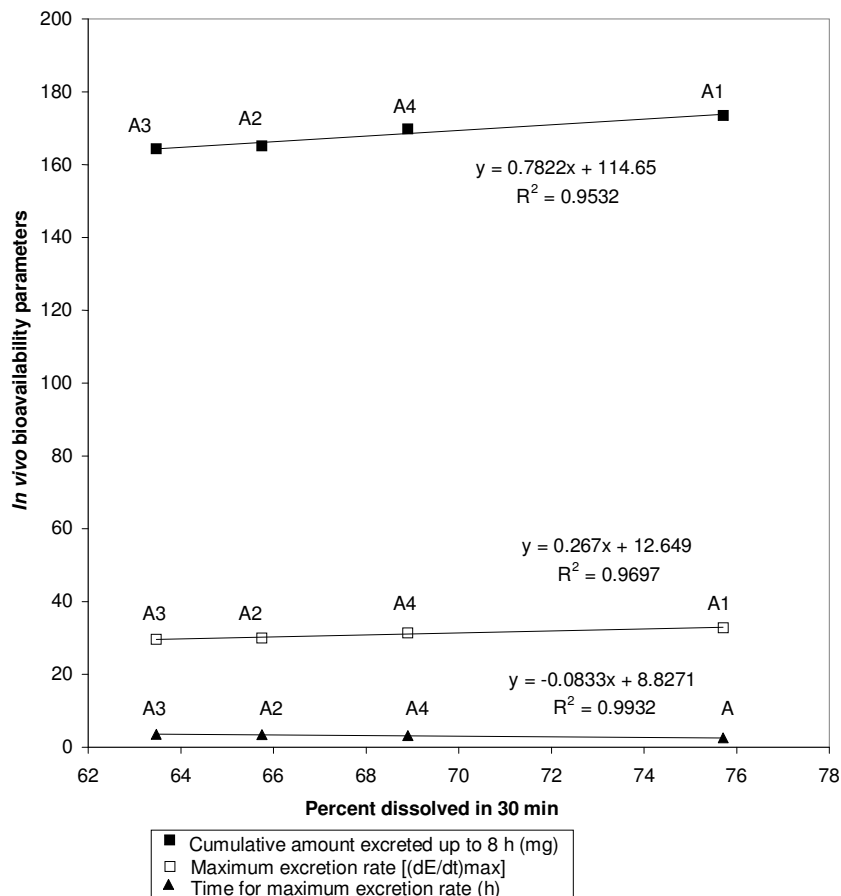
Figure 4. Correlation of *in vitro* dissolution parameter (percent dissolved in 30 min) with various *in vivo* bioavailability parameters.

In vitro-in vivo correlation (IV- IVC)

When various dissolution parameters were correlated with various bioavailability parameters, no significant IV-IVC could be established using E_{24} value. However, significant IV-IVC was observed when cumulative amount excreted up to 8 h (E_8) was employed (Figures 4 to 6) with correlation coefficients $r^2 > 0.8$. As shown in Figure 4, good quantitative correlation coefficients were observed between percent dissolved in 30 min and: (a) Cumulative amount excreted up to 8 h (E_8) ($r^2 = 0.9532$, p < 0.05); (b) Maximum excretion rate $(dE/dt)_{max}$, $r^2 = 0.9697$, p < 0.05) and (c) Time for maximum excretion rate (T_{max}) ($r^2 = 0.9932$, p < 0.05), thus the *in vitro* parameter correlated well with the *in vivo* parameters.

Similarly, from Figure 5, good quantitative correlation coefficients were also observed between dissolution rate constant (k) and T_{max} ($r^2 = 0.9813$, p < 0.05). With regards to E_8 and $(dE/dt)_{max}$, the correlation was not as perfect as that of T_{max}, with correlation coefficients just above 0.8, with exact values of 0.8564 and 0.8879, respectively.

Finally, Figure 6 equally shows that good quantitative correlations were established between time for 50%

dissolution ($DT_{50\%}$) and (a) E_8 ($r^2 = 0.9041$, p < 0.05), (b) $(dE/dt)_{max}$ ($r^2 = 0.9297$, p < 0.05) and (c) T_{max} ($r^2 = 0.9954$, p < 0.05)

In all the IV-IVC performed, best correlation was obtained between the *in vivo* parameter T_{max}, and all the *in vitro* parameters used for the correlation. The correlation coefficient recorded in each case was higher than others. The significant IV-IVC observed were in agreement with the observations of some workers (Young et al., 1997; Hussein and Friedman, 1990) who carried out their own studies on aspirin in centers outside Nigeria.

Conclusion

Good *in vitro-in vivo* correlations were established between various dissolution and bioavailability parameters of four commercial brands of aspirin tablets using Level C *in vitro-in vivo* correlation approach. Therefore, with proper standardization of methods of assessment, *in vitro* dissolution parameters can be used to predict *in vivo* bioavailability of these aspirin tables marketed in Nigeria.
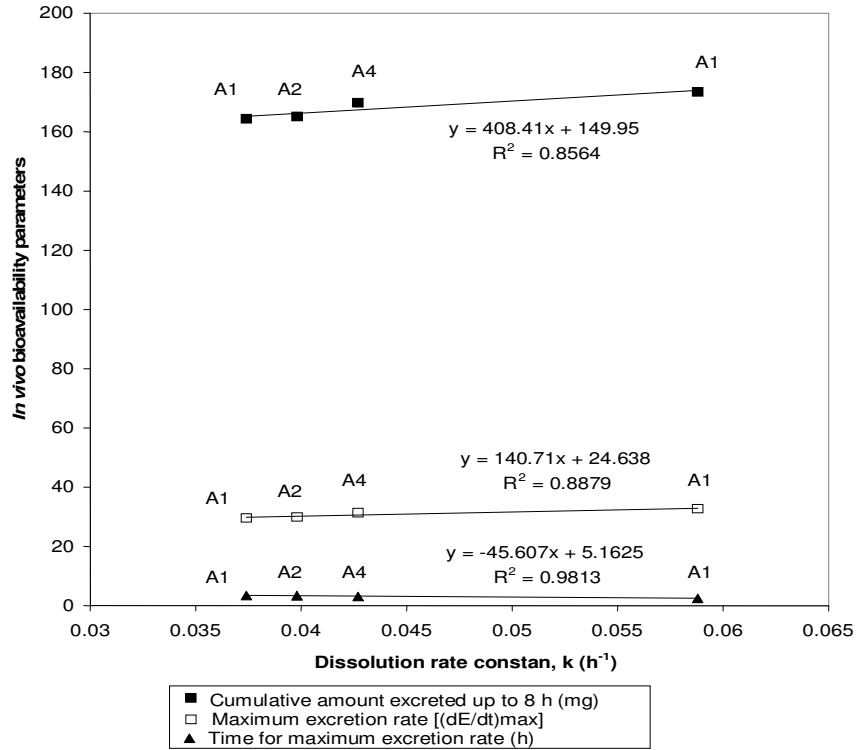
Figure 5. Correlation of *in vitro* dissolution rate constant with various *in vivo* bioavailability parameters.
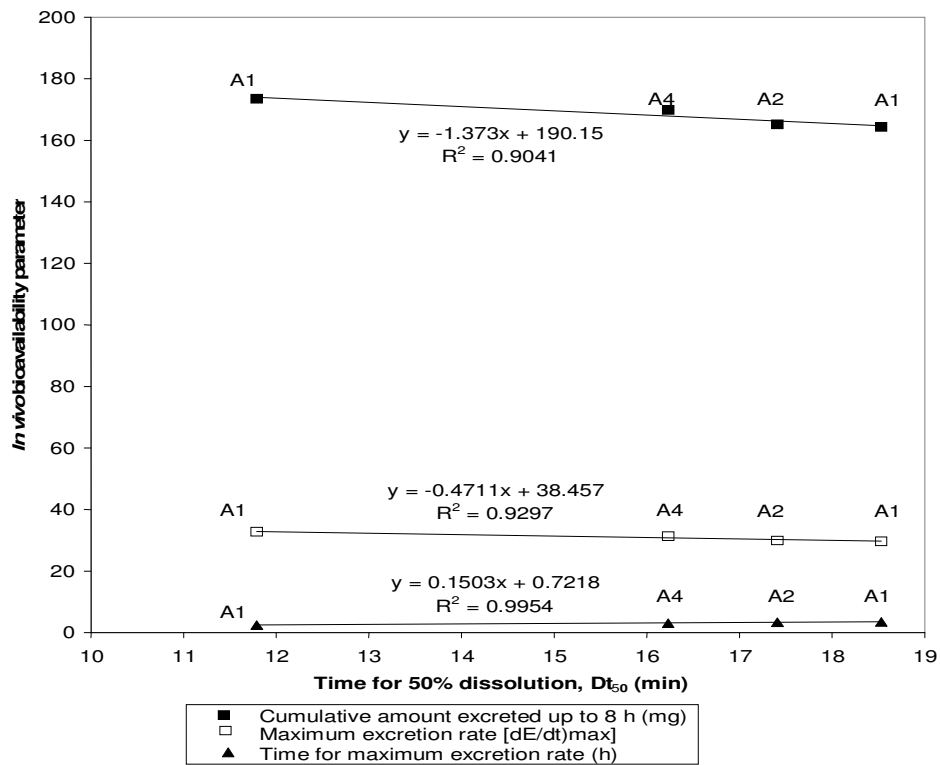

Figure 6. Correlation of time for 50% *in vitro* dissolutiion with various *in vivo* bioavailability parameters.

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