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Correlation of 'in vitro' release and 'in vivo' absorption characteristics of rifampicin from ethylcellulose coated nonpareil beads

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Abstract

The purpose of this study was to investigate the possibility to develop different levels of correlation between in vitro dissolution parameters and in vivo pharmacokinetic parameters for three rifampicin formulations. A level A correlation of in vitro release and in vivo absorption could be obtained for individual plasma level data by means of the Wagner and Nelson method. Linear correlation could be obtained when percent dose released in vitro was plotted vs percent dose absorbed in vivo with correlation coefficients between 0.954, 0.983 and 0.997 for the formulations studied. A second level correlation between mean in vitro dissolution time (MDT) and mean in vivo residence time (MRT) was performed with a correlation coefficient of 0.536, 0.420 and 0.335. Finally, it was also possible to establish a good in vitro–in vivo correlation when the $T_{50\%hrs}$ (time taken to release 50% of rifampicin) in vitro and C_{max}, T_{max} or AUC in vivo were compared.

Keywords: Rifampicin; Release in vitro; Absorption in vivo; In vitro–in vivo correlation

1. Introduction

Controlled release dosage forms are becoming increasingly important either to achieve the desired levels of therapeutic activity required for a new drug entity or to extend the life cycle of an existing drug through improved performance or patient compliance. A fundamental question in evaluating a controlled release product is whether formal clinical studies of the safety and efficacy of the dosage form are needed or whether a pharmacokinetic evaluation will suffice.

The in vitro dissolution test is important for the purpose of: (a) providing necessary process control; (b) stability determination of the release rate characteristics of the product; and (c) facilitating certain regulation determinations and judgments concerning minor formulation changes (Skelley et al., 1990). Correlation between in vitro testing and in vivo performance are encouraged and guide-

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lines were published in the proceedings of a controlled release workshop (Blume et al., 1995) and a chapter about in vitro and in vivo evaluation of the dosage forms is included in USP 23 (1995).

In vitro in vivo correlation (IVIVC) is a predictive mathematical model describing the relationship between an in vitro property of a dosage form, usually the rate or extent of drug dissolution or release, and a relevant in vivo response e.g. plasma drug concentration or amount of drug absorbed. To obtain an IVIVC, at least three batches of the same drug should be available which differ in their in vivo as well as in vitro performance. The IVIVC guidelines presents a comprehensive perspective on (1) methods of developing an IVIVC and evaluating its predictability; (2) using an IVIVC to set dissolution specifications; and (3) applying an IVIVC as a surrogate for in vivo bioequivalence when it is necessary to document bioequivalence during the initial approval process or because of certain pre- or postapproval changes (e.g. formulation, equipment, process and manufacturing site changes).

Rifampicin, the semi synthetic hydrazine derivative of rifampicin B, one of the most potent and powerful mycobactericidal drug is used mainly in intermittent therapy, both in tuberculosis and leprosy (Girling and Hitze, 1979; Jopling, 1983). The biological half-life varies from 2 to 5 h (Martindale, 1993). The prolonged treatment of tuberculosis with conventional chemotherapy of rifampicin and its adverse side effects promoted the development of controlled release formulations.

In the last several years many different types of rifampicin controlled release formulations have been developed to improve clinical efficacy of drug and patient compliance (Mathur et al., 1985; Uppadhyay et al., 1997; Schierholz, 1997; Deol and Khuller, 1997; Denkbas et al., 1995; Amar and Khalil, 1997; Nakhare Sushma and Vyas, 1995; Khopade et al., 1996; Barik et al., 1993). In the present investigation we prepared ethylcellulose coated nonpareil beads and developed in vitro in vivo correlations at different levels.

Table 1
Composition of drug layering solution

Ingredients	Percentage
Rifampicin	10% w/v
Polyvinyl pirrolidine K-30	0.25% w/v
Dichloromethane:methanol (4:1)	100 ml

2. Materials and methods

2.1. Materials

Rifampicin I.P. was obtained from M/s. Lupin Laboratories, India; ethylcellulose (S.D. Fine Chem. Ltd; India), nonpareil starch beads gift from Natco Pharma Ltd. India, propylene glycol, methanol (Qualigen Fine Chemicals, India) dichloromethane (Qualigen Fine Chemicals, India); acetone (Qualigen Fine Chemicals, India); chloroform (Qualigen Fine Chemicals, India); disodium hydrogen phosphate (S.D. Fine Chem. Ltd; India), polyvinyl pirrolidone K-30 (PVP K-30) gift sample from VECO pharma, India, sodium hydroxide (Qualigen Fine Chemicals, India); potassium dihydrogen orthophosphate (S.D. Fine Chem. Ltd; India), and ascorbic acid (S.D. Fine Chem. Ltd; India).

2.2. Drug layering

Approximately 2500 g of nonpareil beads (mesh No. 22/24) were used as initial cores for drug loading. Table 1 shows the coating composition used for drug layering. Rifampicin & PVP K-30 were dissolved in dichloromethane–methanol mixture and sprayed radially using spray gun under pressure onto the rotating nonpareil bead in the coating pan. PVP K-30 was used as a binder. Methanol and dichloromethane mixture was taken as a solvent for easy drying after drug layering.

A laboratory size Kalweka (Cadmach Machinery Co. Pvt. Ltd., India) coater was used for loading the drug solution. The flow rate of the solution was maintained constant at 25 ml/min, which prevents the agglomeration of beads during coating process. The inlet air temperature (Silencio 1000 drier, Braun, a division of Gillette, New

Delhi, India) was 45 °C and the drying time after each application was 2 min. The speed of the coating pan was 32 rpm.

2.3. Film coating

Approximately 1000 g of drug loaded beads, were used for film coating. A 5% w/v solution of ethyl cellulose in acetone was prepared. To this 30% w/w of propylene glycol was added with respect to polymer weight. The drug loaded beads were coated using pan coating (Kalweka). The polymer solution was sprayed radially using spray gun (Uniques India Ltd., India) under pressure onto the rotating rifampicin loaded nonpareil beads at the flow rate of 25 ml/min. The inlet air temperature (Silencio 1000 drier, Braun, a division of Gillette, New Delhi, India) was 45 °C and the drying time after each application was $2\frac{1}{2}$ min. The speed of the coating pan was 32 rpm. Samples were taken periodically throughout a run to give different coating thickness. The coating level is the quotient of the dry weight of polymer and the weight of the uncoated drug loaded beads, expressed as a percentage. All beads were stored in plastic bags at room temperature until required.

The following formulations were prepared using blends of coated beads.

1. 2:2:1 ratio of 2:3:4% EC coated nonpareil beads.
2. 2:2:1 ratio of 6:4:2% EC coated nonpareil beads.
3. Three percent EC coated nonpareil beads.

2.4. In vitro studies

Ethyl cellulose coated beads equivalents to 300 mg of rifampicin were taken in a basket of the USP XXI dissolution apparatus 1. The stirring rate was 100 rpm pH 7.4 phosphate buffer containing ascorbic acid (200 μg/ml) as used as dissolution medium (900 ml) and was maintained at 37 ± 1 °C. Samples of 5 ml were withdrawn at predetermined time intervals with a pipette fitted with a filter. The collected samples were diluted suitably, if necessary, and were analyzed for the rifampicin content by UV spectrophotometric method at 475 nm. The volume withdrawn at each time interval was replaced with fresh quantity 5 ml of dissolution medium. Each dissolution study was performed in three times and mean values were taken.

At the end of 12 h of dissolution testing, the remaining beads were collected and suspended in methanol and the remaining drug content was estimated. This is to make sure that the amount of drug remaining adds to the drug release to give total mass balance of drug content.

2.5. In vivo studies

In vivo studies were done on the following products:

1. Rifampicin powder + 2% sodium starch glycolate (filled in hard gelatin capsule).
2. 2:2:1 ratio of 2:3:4% ethylcellulose coated nonpareil beads (filled in hard gelatin capsule).
3. 2:2:1 ratio of 6:4:2% ethylcellulose coated nonpareil beads (filled in hard gelatin capsule).
4. Three percent EC coated nonpareil beads (filled in hard gelatin capsule).

In vivo experiments were carried out in healthy human volunteers as per the following experimental design and protocol.

2.5.1. Experimental design

A Latin Square Design (LSD) with $n = 4$ was chosen to test the selected treatments. In this design each subject receives each formulation, but, in addition, each treatment occurs once in every 15 days such that all products are tested in all the four volunteers during the study.

2.5.2. In vivo study protocol

Human subjects of weight 50–65 kg of male sex age 22–25 years participated in this study. Subject's liver and kidney functions were assessed to be normal by clinical and standard biochemical investigations. None of the subject is on alcohol or tobacco. The subjects refrained from all medication for 15 days prior to and during the course of study. All the participants have given written consent and the Institutional Review Board approved the protocol of the study. The subjects were fasted over night at least 10 h prior to dose.

After collecting the zero hour blood sample (blank), the product involved in the study was administered orally at a dose equivalent to 300 mg of rifampicin with 200 ml of water. Food was withheld for a period of 2 h. Five milliliters of blood was withdrawn from a cubital vein with a heparinized syringe at 1–6, 8, 10, 12, 18, and 24 h after administration of the product. Blood samples were centrifuged immediately at 5000 rpm and the separated plasma samples were stored at −20 °C until analysis. Volunteers were provided with a standard breakfast and a standard vegetarian lunch 4 h after breakfast. Rifampicin content of the plasma samples was determined by known high performance liquid chromatographic method (Mika Ishii and Hiroyasu Ogata, 1988). The chromatographic system consisted of a Model 2800 Bio Rad Solvent Delivery System; samples were chromatographed at room temperature on a reversed phase Bio Sil ODS-55 (catalog 125-0080, 250 × 4 mm) column. The mobile phase consisting of methanol: 0.01 M disodium hydrogen phosphate (30:70) was used at a flow rate of 0.8 ml/min and the pressure was approximately 195–200 kg/cm^2. A guard column (Bio Rad Model-1250131) was used.

To a 0.5 ml aliquot of sample (plasma sample) was added 2 ml of 0.5 M sodium dihydrogen phosphate. The mixture was shaken mechanically with 7 ml of chloroform for 10 min. After centrifugation at 3000 rpm for 15 min, the upper aqueous phase was aspirated off using syringe and 5 ml of the lower organic phase were taken to dryness at 40 °C under reduced pressure. The residue was dissolved in 0.5 ml of the mobile phase and 20 μl of the solution was injected into the HPLC column. Ultraviolet absorption was measured at 340 nm at 0.0025 a.u.f.s., using Bio Rad UV monitor model 1306.

From time versus plasma concentration data, various pharmacokinetic parameters such as peak concentration (C_{max}), time at which peak occurred (T_{max}), area under the curve (AUC) and mean residence time (MRT) were calculated in each case according to noncompartmental approaches from the usual relationships (Gibaldi and Perrier, 1982). The in vivo percent of drug absorbed was calculated by Wager–Nelson method. For calculating the absorption rate constants, one compartment model was used.

2.5.3. Determination of the percentage absorbed to various times and absorption rate constant (K_a)

Percentage absorbed at various times was calculated from plasma concentration data by the method described by Wagner and Nelson. The equation developed for the determination of absorption rate from blood concentration data is:

$$\frac{dA}{dt} = V_d \frac{dC_b}{dt} + K_{el}\, C_b\, V_d,$$

where dA/dt is the absorption rate, V_d is the apparent volume of distribution, dC_b/dt is the rate of change of blood concentration (C_b) at time t and K_{el} is elimination rate constant.

The equation may be integrated between the limits of $t = 0$ and $t = T$ and divided by V_d to give,

$$\frac{A_T}{V_d} = C_T + K_{el} \int_{t=0}^{t=T} C_b dt$$
$$= C_T + K_{el}(AUC)_{t=0-T},$$

where A_T is the amount of drug absorbed to time t, C_T is the blood concentration at $t = T$ and the quantity under the integral sign is the area under the blood level versus time curve between the indicated limits. When the successive values of A_T/V_d are calculated a maximum or asymptotic value $(A_T/V_d)_\alpha$ is obtained. The maximum or asymptotic value is divided into successive values of A_T/V_d to yield percentage absorbed data i.e.:

$$\left[\frac{(A_T/V_d)}{(A_T/V_d)_\alpha} \times 100\right] \text{ as a function of time.}$$

The plot of log percent unabsorbed versus time gives straight line and the slope of the line is equal to $-K_a/2.303$. The absorption rate constant (K_a) was calculated from the slope of the line.

2.6. In vitro–in vivo correlation (IVIVC)

Three levels of correlation have been defined according to the USP 23 (1995).

2.6.1. Level A correlation: correlation between percent absorbed in vivo versus percent released in vitro

The in vitro dissolution curve of the product is compared with the in vivo absorption curve generated by Wagner–Nelson method. The mean data for the in vivo percent absorbed were plotted versus time and the in vitro drug released versus time were superimposed on the first plot. However, the simplest way to demonstrate a correlation is to plot the fraction absorbed in vivo (obtained by Wagner–Nelson method) versus the fraction released in vitro at the same time.

2.6.2. Level B correlation: correlation between mean in vitro dissolution time and mean in vivo residence time

In this level of correlation, the mean in vitro dissolution time of the product is compared with either the mean in vivo residence time (MRT) or the mean in vivo absorption time (MIT) or compared with the in vitro dissolution rate constant to the absorption rate constant.

2.6.3. Level C correlation: correlation between the in vitro dissolution parameters and in vivo pharmacokinetic parameters

In this level of correlation a mean in vitro dissolution time or the amount dissolved at a particular time or the time required for in vitro dissolution of a fixed percent of the dose e.g. $T_{50\%}$ is compared with different pharmacokinetic parameters obtained from in vivo studies. The in vivo pharmacokinetic parameters used for correlation were the half-life of elimination ($t_{\frac{1}{2}}$); maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}) and the area under the plasma levels curve (AUC).

2.6.4. Mean in vitro dissolution time (MDT)

The mean time for the drug to dissolve under in vitro dissolution conditions. This is calculated using the following equation.

$$\mathrm{MDT} = \frac{\int_0^\infty M_\infty - M(t)\,\mathrm{d}t}{M_\infty}, \qquad (6)$$

where, M_∞ is initial amount of dose and $M(t)$ is amount of the drug released at time t. MDT vitro was defined as the time when 50% of the drug had been released since the in vitro dissolution could be approximated to zero order kinetics.

3. 3. Results and discussion

Percent of drug loaded on nonpareil beads was found to be 52%. Fig. 1 shows the evolution of the average plasma levels of rifampicin after the oral administration of pure rifampicin and three tested formulations. The elimination rate constant K_{el}, for rifampicin was found to be 0.1583 h^{-1} and the corresponding biological half-life ($t_{\frac{1}{2}}$) was found to be 4.38 h following the oral administration of rifampicin in solid form. The $t_{\frac{1}{2}}$ value of rifampicin obtained in the present work is in good agreement with the earlier reported value of 2–5 h (Martindale, 1993; Tripathi, 1995). The pharmacokinetic parameters determined by noncompartmental model are listed in Table 2.

The dissolution profiles (in vitro) were compared with the cumulative absorption profiles (in vivo) obtained by using Wager–Nelson method of the plasma levels for the three formulations studied, shown in Fig. 2. The rates of release and absorption for these formulations followed first order kinetics (figures not shown). The results thus indicated that rifampicin from coated beads was released and absorbed slowly over longer periods of time which in turn maintained the plasma concentration within the therapeutic index (0.1–7 μg/ml) over longer periods of time.

An IVIVC should be evaluated to demonstrate the predictability of in vivo performance of a drug product from its in vitro dissolution characteristics to maintain over a range of in vitro dissolution release rates and manufacturing changes. Since the objective of developing and IVIVC is to establish a predictive mathematical model describing the relationship between an in vitro property and a relevant in vivo response.

The main objective of developing and evaluating an IVIVC is to establish the dissolution test as a surrogate for human bioequivalence studies, which may reduce the number of bioequivalence

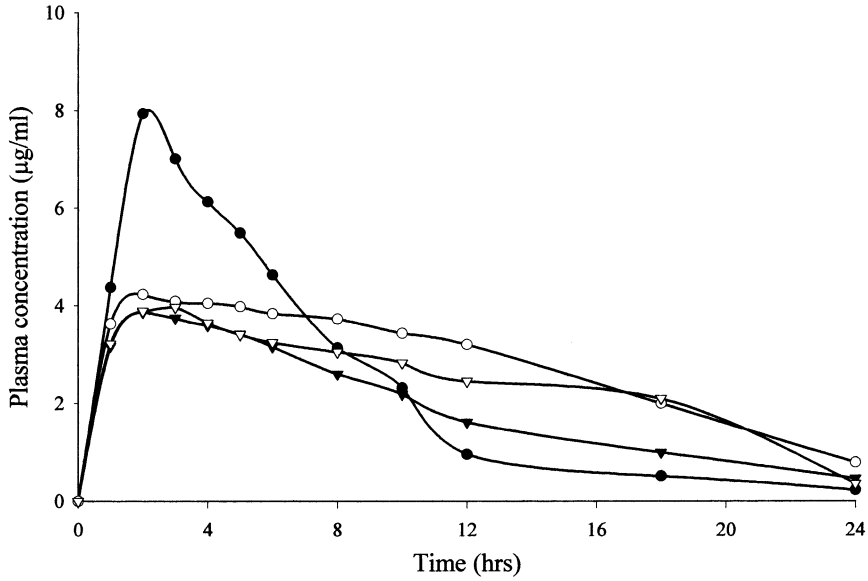

Fig. 1. Plasma concentrations of rifampicin following its oral administration ($n = 4$) -●- pure drug; -○- 2:2:1 ratio of 2:3:4% EC; -▼- 3% EC; -▽- 2:2:1 ratio of 6:4:2% EC.

Table 2
Summary of the pharmacokinetic parameters estimated following the oral administration of rifampicin in pure solid form and in ethylcellulose coated nonpareil beads

S. No.	Product	C_{max} (µg/ml)	T_{max} (h)	(AUC) (µg h/ml)	MRT (h)	K_{el} (h^{-1})	K_a (h^{-1})	$T_{50\%}$
1	Rifampicin (solid form)	7.94 ± 2.54	2	56.365 ± 21.54	6.184 ± 1.52	0.1583 ± 0.02	3.2088 ± 0.84	
2	2:2:1 ratio of 2:3:4% EC coated nonpariel beads	4.23 ± 1.05	2	67.29 ± 20.58	9.666 ± 3.80	0.0239 ± 0.003	1.0615 ± 0.52	1.657
3	2:2:1 ratio of 6:4:2% EC coated nonpareil beads	3.97 ± 0.98	3	45.99 ± 19.74	8.632 ± 2.21	0.0263 ± 0.001	0.7939 ± 0.25	3.148
4	3% EC coated nonpareil beads	3.87 ± 1.02	2	52.23 ± 25.60	8.445 ± 2.75	0.02856 ± 0.002	0.6574 ± 0.27	1.407

studies performed during the initial approval process as well as with certain scale-up and postapproval changes.

Linear correlation plots for percentage of in vivo dose absorbed and percent of in vitro dose released are shown in Fig. 3. An acceptable correlation was obtained in ethylcellulose coated beads with a good linear-fitting with correlation coefficients of 0.954, 0.983 and 0.997 for 2:2:1 ratio of 2:3:4% EC coated beads, 2:2:1 ratio of 6:4:2% EC coated beads and 3% EC coated beads. This kind of correlation is quite important since it repre-

sents a point to point relationship between in vitro dissolution and the in vivo input rate of the drug from the dosage form. Thus an in vitro dissolution curve can serve as surrogate for in vivo performance.

Poor correlation coefficients between in vitro mean dissolution time values and in vivo mean residence time values were observed. The correlation coefficient values are 0.536, 0.420 and 0.335 for 2:2:1 ratio of 2:3:4 EC; 6:4:2% EC and 3%EC coated beads, respectively.

A good correlation was observed between $T_{50\%}$ versus $T_{max,}$ C_{max} and AUC with corresponding coefficient values 0.991, 0.990 and 0.911, respectively.

The last type of correlation represents a single point correlation. It does not reflect the complete shape of the plasma level profile, which is the critical factor that defines the performance of modified release products. Since this type of correlation is not predictable of actual in vivo product performance, it is generally only useful as a reference in formulation development or as a product quality control reference procedure.

Significant correlation between the in vitro and the in vivo parameters indicates that the in vitro release rate procedure is capable of discriminating between extended release formulations having different in vivo bioavailabilities. A level A IVIVC is considered to be the most informative and is recommended for regulatory purposes. Level C

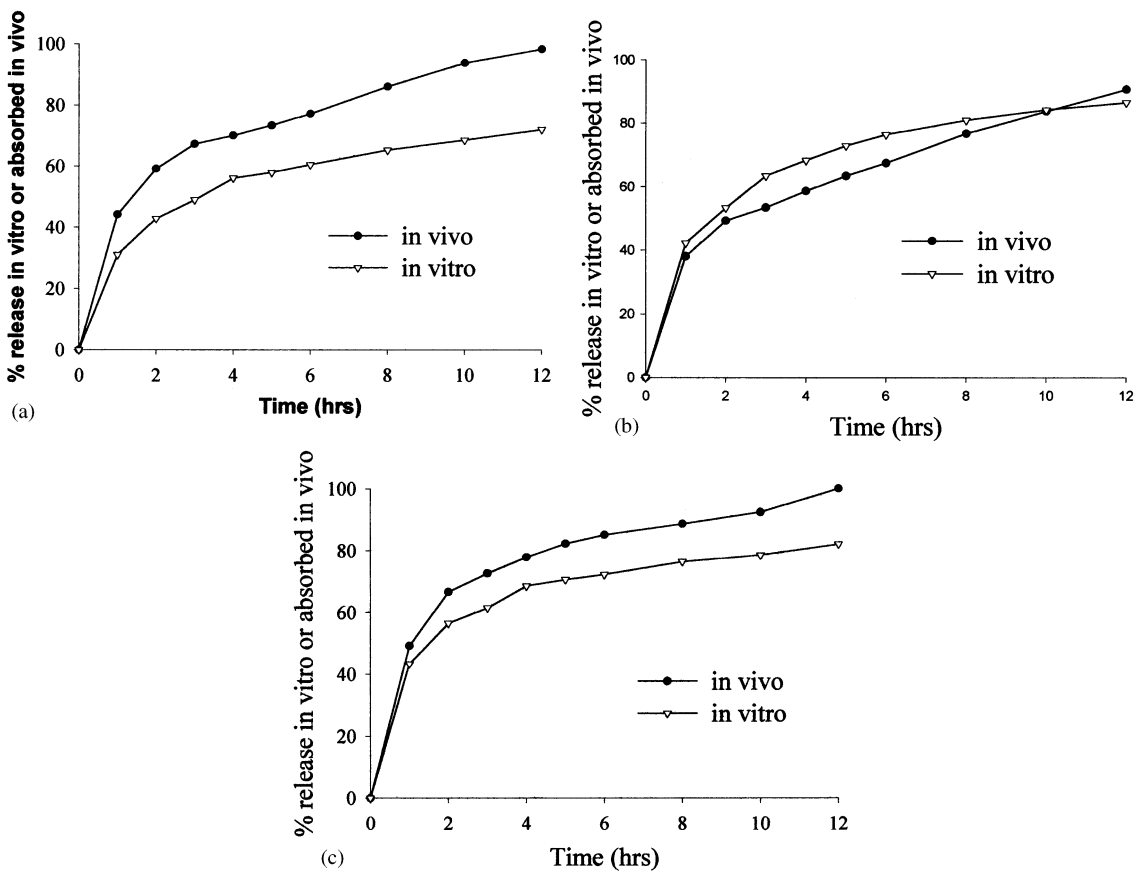

Fig. 2. (a) Comparison of the in-vitro release profiles and cumulative in-vivo absorption profiles of 2:2:1 ratio of 6:4:2% EC. (b) Comparison of the in-vitro release profiles and cumulative in-vivo absorption profiles of 2:2:1 ratio of 2:3:4% EC. (c) Comparison of the in-vitro release profiles and cumulative in-vivo absorption profiles of 2:2:1 ratio of 3% EC.

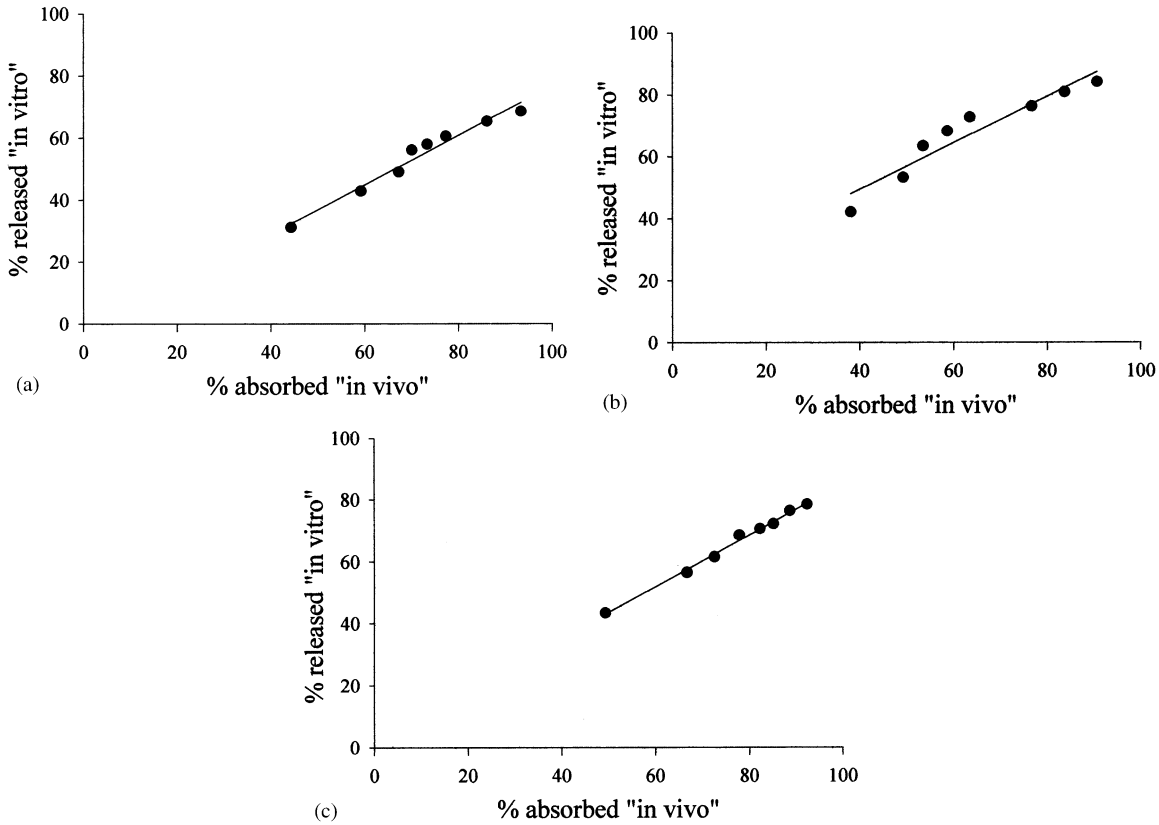

Fig. 3. (a) Plots of mean percentage of dose absorbed in-vivo versus mean percent released in-vitro for 2:2:1 ratio of 6:4:2% EC coated nonpareil beads (propylene glycol as plasticizer). (b) Plots of mean percentage of dose absorbed in-vivo versus mean percent released in-vitro for 2:2:1 ratio of 2:3:4% EC coated nonpareil beads (propylene glycol as plasticizer). (c) Plots of mean percentage of dose absorbed in-vivo versus mean percent released in-vitro for 2:2:1 ratio of 3% EC coated nonpareil beads (propylene glycol as plasticizer).

correlation can be useful in the early stages of formulation development, when pilot formulations are being selected. Level C correlation is least useful for regulatory purposes. Level B and Level C correlation guidelines described by FDA/AAPS (Food and Drug Administration/American Association of pharmaceutical Sciences) and USP 23, provide the manufacturers with a valuable in vitro test that can be used to obtain information on the in vivo absorption behavior of such formulations.

In the present in vitro in vivo correlation methods, level A correlation have given best correlation coefficients than level B and level C. This type of correlation represents a point-to-point relationship between in vitro dissolution and in vivo input rate. A level A correlation is considered to be most informative than other levels of correlation.

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