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In Vitro-In Vivo Correlation of Different Modified Release Formulations of Theophylline

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Using the *in vitro* dissolution data and bioavailability data we present our results of *in vitro-in vivo* correlation of different modified release formulations of theophylline. As part of our ongoing study an experimental modified release capsule formulation, containing theophylline (200 mg)-loaded microspheres (Formulation F4), was developed, characterised and its *in vitro* and *in vivo* performance was then compared with that of the three market modified release formulations of theophylline (200 mg) - two tablets (Formulations F2 and F3) and one capsule (Formulation F1). Formulations F1, F2 and F3 were analysed to find out the best market sample with acceptable bioavailability. All the four formulations were evaluated for *in vitro* theophylline release using different dissolution test conditions. Pharmacokinetic evaluation of these formulations was carried out in six healthy volunteers and the parameters were established using non-compartmental analysis. *In vitro-in vivo* correlations were established from the generated dissolution and bioavailability data. *In vitro* studies indicated that only formulation F1 showed pH-dependent drug release while the other three formulations, including experimental formulation F4, showed almost condition-independent dissolution behaviour. The bioavailability studies indicated that amongst the market formulations (F1, F2, F3), formulations F1 and F2 were bioequivalent but F3 failed to demonstrate acceptable dissolution and bioavailability. Thus, switchability of formulation F3 for formulations F1 or F2 is questionable. A good correlation (R^2=0.9986, slope=1.0614 and intercept=0.1824, supporting Level A correlation) was observed for microsphere formulation, F4, under conditions of Test # 1. Although experimental formulation F4 showed acceptable dissolution behavior and bioavailability, it did not, however, exhibit bioequivalence with two other market samples, either F1 or F2. Dissoluton conditions of Test # 1 (dissolution medium, pH 1.2 simulated gastric fluid without pepsin for 1 h followed by pH 6 phosphate buffer for rest period) reflects highly significant correlation (R^2 > 0.98) with corresponding bioavailability for all the four formulations.

Presently dissolution test is the most meaningful and important *in vitro* test for quality control of solid dosage forms. When carried out in a suitable medium under appropriate and reproducible conditions, the dissolution test can show a meaningful correlation with bioavailability of a drug formulation. Correlation between *in vitro* dissolution and *in vivo* bioavailability cannot be taken for granted but must be demonstrated convincingly to guarantee batch-to-batch reproducibility in biological performance of a formulation[1].

Meaningful correlation of *in vitro* dissolution and *in vivo* absorption of the drug (IVIVC) assumes importance for the process control of formulations of drugs having narrow therapeutic window and hence requiring close drug level monitoring[1]. This is especially of significance for designing modified release (MR) [sustained release (SR)/extended release (ER)/controlled release (CR)] formulations of such drugs[1,2].

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The need to establish and utilize IVIVCs for the assessment of MR dosage forms is assuming importance. The guidelines are available for development of correlations of different levels of significance namely Level A, B and C correlations in decreasing order of their utility. Level A correlations are deemed to provide the most useful biopharmaceutical information[2].

Theophylline (THP) inhibits adenosine-induced bronchoconstriction and is used commonly as a bronchodilator in chronic obstructive pulmonary disease. It has an elimination half-life of 5-9 h in non-smoking healthy volunteers[3]. It has a narrow therapeutic window (therapeutic concentration in plasma, 5 to 15 μg/ml[4]) and hence, needs therapeutic monitoring of the drug levels in the body. It is rapidly, consistently and completely absorbed when administered in solution form; peak plasma levels are attained within 2-3 h[3,5]. Several reports have appeared describing approaches to establish correlation between *in vitro* dissolution and *in vivo* absorption of THP[6-11] and other drugs[12]. All these attempts address different types of IVIVCs, which are product specific. This study describes IVIVC for different MR formulations of THP (tablets and capsules, with different composition and varying process of manufacture).

As a part of our continuing formulation development programme, a modified release capsule formulation, containing THP-loaded microspheres, was developed and characterized for its *in vitro* dissolution performance and the batch with optimum dissolution performance was studied for *in vivo* bioavailability. Since drug release from MR formulations is controlled by the composition and the engineering of the formulation, USP 24 prescribes various *in vitro* dissolution test conditions for theophylline extended release capsule formulations. It also emphasizes that the product label should state the dissolution test conditions with which the product complies[13]. Though not official in IP[14], several MR formulations of THP are available in the Indian market. Therefore, this study was undertaken to study the experimental formulation and three market samples of MR formulations of theophylline, for their dissolution behaviour under different dissolution test conditions. The dissolution behaviour and bioavailability of THP from the experimental formulation developed in our laboratory was compared with that of the marketed MR formulations of THP. Relevance of *in vitro* dissolution results with the bioavailability of THP from these four formulations was evaluated by establishing meaningful *in vitro-in vivo* correlations (IVIVCs).

In this study three marketed MR formulations containing 200 mg of THP, two tablets (F2, F3) and one capsule (F1) formulation and an experimental capsule formulation (F4) containing ethyl cellulose microspheres loaded with equivalent amount of THP (200 mg) developed in the laboratory, were tested for their *in vitro* dissolution characteristics under different dissolution conditions and *in vivo* absorption pattern in healthy human volunteers. The market formulations were included in the study in order to select sample/s with acceptable bioavailability of THP for bioequivalence asessment. Cumulative percentage (%) of THP absorbed was calculated using Wagner-Nelson method[15] and correlated with cumulative percentage of THP dissolved *in vitro*.

MATERIALS AND METHODS

Analytically pure anhydrous theophylline was gifted by Core Healthcare Ltd., Ahmedabad. Ethyl cellulose (viscosity of 5% w/w solution in 80:20 toluene:ethanol by weight at 25° = approximately 14 cpi, degree of substitution - 2.42 to 2.53, Central Drug House, Mumbai), acetone, petroleum ether (60-80° fraction), heavy paraffin (LR, S. D. Fine-Chem Ltd., Boisar), dioctyl sodium sulphosuccinate (DOSS, National Chem., Vadodara) were used for preparation of microsphere formulation. Potassium dihydrogen phosphate, disodium hydrogen phosphate, hydrochloric acid (33% v/v), sodium hydroxide, orthophosphoric acid (LR, S. D. Fine-Chem Ltd., Boisar), methanol, tetrahydrofuran (HPLC, Spectrochem, Mumbai) and triple glass-distilled water were used for preparation of dissolution media and HPLC analysis. Dissolution samples were analyzed for the content of THP at 271 nm using a Shimadzu 160A double beam UV/Vis spectrophotometer using the standard calibration curve method.

Formulations:

An experimental capsule formulation (F4) was prepared by filling ethyl cellulose microspheres (size range 355-500 mm), loaded with THP equivalent to 200 mg of THP, in a hard gelatin capsule. The established brands of MR formulations obtained from market included one capsule (F1) and two tablet dosage forms (F2 and F3) containing 200 mg of THP per capsule or tablet.

Preparation of THP microspheres:

Several batches of THP microspheres with different drug:polymer ratio (D:P; 1:1, 2:1, 2:1.25 w/w) were prepared using emulsification-solvent evaporation technique, where dispersion of THP in acetone solution of ethyl cellulose was poured into a 0.05% w/w solution of DOSS in heavy paraffin

as the continuous phase. Microspheres, thus prepared, were subjected to analysis of size distribution, determination of drug content and dissolution studies. *In vitro* dissolution studies of microspheres with different D:P ratio and microspheres of different diameters were carried out by using 0.1 N HCl for 1 h followed by pH 7.5 buffer upto 9 h at 50 rpm using USP dissolution test apparatus 1 (basket) after filling the microspheres equivalent to 200 mg of THP into a hard gelatin capsule (size 0).

In vitro dissolution studies:

The dissolution testing was performed for each MR formulation using either rotating basket (for capsules) or rotating paddle (for tablets) (USP Dissolution Test Apparatus 1 and 2, respectively). The dissolution medium was maintained at 37±0.5°. The dissolution test conditions are summarized in Table 1. A dissolution sample of 5 ml was removed from each vessel at the predetermined time intervals (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 h when required) and was replaced by equal volume of corresponding dissolution medium. These samples were analyzed by UV/Vis spectrophotometer at 271 nm after appropriate dilutions with the same dissolution medium and concentration of THP was calculated by reference to the calibration curves constructed from reference standards prepared in dissolution medium of relevant dissolution study.

In vivo study of MR formulations of THP:

Six healthy non-smoking male volunteers ranging in age 20 to 25 y and weighing 50 to 55 kg participated in the study. The written informed consent was obtained from all the volunteers and the study protocol was approved by an Independent Ethical Committee (IEC). None of the volunteers received any other drug one week prior to study and during the study. The volunteers abstained from consuming any xanthine containing food or drinks (chocolates, tea, coffee, coke) for 48 h before administration of the formulation and throughout the study. They were fasted overnight and fasting was continued until 4 h post dose, but water intake was not restricted. Each volunteer received single dose of each formulation in a randomized four-way cross-over design with a wash out period of 8 d between two treatments. Blood samples were withdrawn before administration of formulation and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h after administration. The samples were collected in heparinized tubes and centrifuged (3500 rpm, 20 min) to separate plasma. Separated plasma was transferred into polypropylene screw-capped tubes, labeled and stored at -20°, until analysis and were protected from exposure to light. These samples were then analyzed for THP levels by validated isocratic HPLC method[16], using 8-chlorotheophylline as an internal standard.

Bioavailability assessment:

The individual subject plasma THP concentration-time data for treatments F1, F2, F3 and F4 were fitted to noncompartmental analysis using the nonlinear regression program WinNonlin-ProÔ[17] and various pharmacokinetic parameters viz. area under plasma concentration-time curve for first 24 h (AUC_{0-24}), the peak plasma concentration (C_{max}) and the time at which C_{max} is achieved (t_{max}), elimination rate constant (K_{el}), terminal elimination half life ($t_{1/2}$) and area under plasma concentration-time curve extrapolated to infinite time (AUC_{0-}¥), were determined.

In vitro-in vivo correlations (IVIVCs):

In vitro dissolution data for all the four formulations were obtained according to the procedure described earlier (under *in vitro* dissolution studies). Since neither intravenous data nor oral solution data were available for THP, the approach based on Wagner-Nelson method was utilized to achieve an IVIVC. The Wagner-Nelson function, expressed as percentage of the dose absorbed, was calculated using following equation and correlated to the cumulative percentage dissolved *in vitro*[15,18], % dose absorbed = $[(C_t + K_{el}.AUC_{0-t})/(K_{el}.AUC_{0-}¥)]$ 100. Where, C_t is the plasma concentration of drug at time 't' and AUC_{0-t} is the area under the curve from time zero to time 't'.

Using this equation and the data obtained, absorption profiles were constructed for the MR capsules and tablets. The % dose absorbed *in vivo* was plotted against % THP dissolved (released) *in vitro* to obtain an IVIVC for individual formulation.

RESULTS AND DISCUSSION

Several batches of THP microspheres with different drug:polymer (D:P) ratios were prepared (1:1, 2:1 and 2:1.25 w/w). Microspheres with D:P ratio of 2:1.25 w/w showed optimum release under dissolution conditions of Test # 7 (fig. 1a). The microspheres were analysed for THP content and subjected to size distribution analysis by using standard sieves. Effect of microsphere size (250-355 µm, 355-500 µm and 500-1003 µm) on the release of THP was also studied under same dissolution conditions. Microspheres in the size range of 355-500 µm gave optimum dissolution profile when compared with the dissolution test specifications for THP ER capsules of USP 24 (fig. 1b). Therefore, the capsules were filled with microspheres having D:P ratio of 2:1.25

TABLE 1: DISSOLUTION TEST CONDITIONS EMPLOYED.

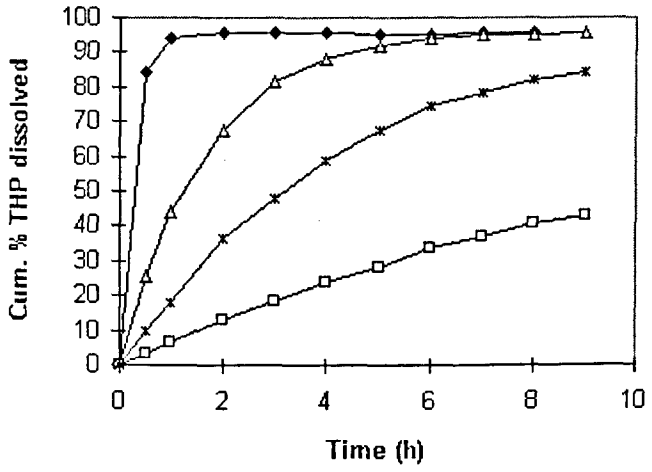
Test #	Dissolution medium (Volume)	Method[a] (Agitation)
1	pH 1.2 SGF[b] (without pepsin) (1 h) followed by pH 6 phosphate buffer (900 ml)	Basket/ Paddle (50 rpm)
2	pH 4.5 phosphate buffer (900 ml)	Basket/ Paddle (75 rpm)
3[c]	0.05 M pH 6.6 phosphate buffer (1000 ml)	Basket/ Paddle (100 rpm)
4	pH 3 phosphate buffer (3.5 h) followed by addition of 5.3 M NaOH to adjust to pH 7.4 ± 0.05 (900 ml)	Basket/ Paddle (50 rpm)
5	pH 3 phosphate buffer (3.5 h) followed by addition of 5.3 M NaOH to adjust to pH 7.4 ± 0.05 (900 ml)	Basket/ Paddle (50 rpm)
6	pH 7.5 SIF[d] (without enzyme) (900 ml)	Basket/ Paddle (100 rpm)
7	0.1 N HCl (1 h) followed by SIF (without enzyme) (900 ml)	Basket/ Paddle (50 rpm)

[a]Basket method was used for capsule formulations while paddle method was used for the tablet formulations; [b]SGF=simulated gastric fluid; [c]Dissolution test conditions for the products labeled for dosing every 24 h; while all other test conditions are prescribed for the products (ER) labeled for dosing every 12 h; [d]SIF=simulated intestinal fluid. Note: The dissolution media, volumes and agitation intensities are as per the monograph through Supplement 3 of USP 24, NF 19 (Theophylline extended release capsules monograph).

w/w and size in the range of 355 to 500 µm (formulation F4) and tested under different dissolution test conditions as per Table 1.

Dissolution specifications for different dissolution tests at different time points for extended release theophylline capsule formulation, recommended by USP 24 and mean cumulative % THP released from F1, F2, F3 and F4 at respective time points, are summarized in Table 2.

In case of capsule formulation F1, the release of THP appeared to be pH-dependent and showed higher release rates as the pH of the dissolution medium increased (fig. 2). It showed faster release in pH 6.6 buffer (Test # 3) and pH



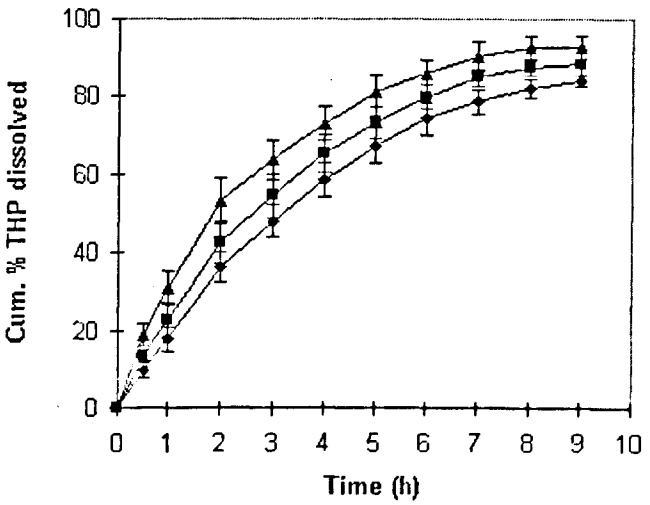


Fig. 1: Effect of D:P ratio and microsphere size on THP release from experimental batches.

a) Effect of drug:polymer ratio [THP:ethyl cellulose; 1:1 (-□-), 2:1 (-Δ-), 2:1.25 w/w (-✳-)] on the release of THP as compared with the conventional capsule formulation [(THP 200 mg, (-◆-)], b) Study of effect of size of microsphere (THP:ethyl cellulose ratio, 2:1.25 w/w) [250-355 µm,(-◆-); 355-500 µm, (-■-); 500-1003 µm, (-▲-)] on the release of THP under conditions of dissolution Test # 7. Each value indicates mean of three observations.

TABLE 2: DISSOLUTION PROFILES OF THEOPHYLLINE MODIFIED RELEASE FORMULATIONS

Average cumulative % THP released [b]						USP specifications [c]
Dissolution test [a]	Time point (h)	Formulation F1	Formulation F2	Formulation F3	Formulation F4	Cumulative % THP released
Test # 1	1	9.656	15.351	12.468	18.776	3-15
	2	41.626	32.503	20.597	45.361	20-40
	4	85.182	56.498	29.617	77.371	50-75
	6	95.225	76.646	36.972	91.505	65.100
	8	96.848	88.009	42.860	95.803	≥ 80
Test # 2	1	12.060	14.106	11.740	21.464	10-30
	2	22.194	31.425	18.659	38.590	35-55
	4	41.138	50.964	27.304	63.680	55-80
	8	65.491	80.272	40.988	86.206	≥ 80
Test # 3	1	55.348	14.091	11.4695	25.727	5-15
	2	76.932	25.466	17.6	46.806	12-30
	4	88.826	46.134	27.879	77.783	25-50
	5	91.613	55.253	31.479	85.240	30-60
	8	93.146	79.598	42.332	100.412	55-75
Test # 4	1	8.525	13.922	9.582	18.691	13-38
	2	18.416	22.981	16.361	39.089	25-50
	3.5	50.343(4h)	42.159 (4h)	29.105 (4h)	70.756 (4h)	37-65
	5	78.977	49.116	33.177	870.738	85-115
Test # 5	1	8.525	13.922	9.582	18.691	10-30
	3.5	50.343(4h)	42.159 (4h)	29.105 (4h)	70.756 (4h)	30-60
	5	78.977	49.116	33.177	80.738	50-80
	7	10.227	62.386	39.189	95.125	≥ 65
	10	99.069	76.133	47.33	101.107	≥ 80
Test # 6	1	65.328	16.921	12.377	21.014	3-30
	2	82.643	28.039	18.207	37.173	15-50
	4	92.742	49.477	26.167	61.330	45-80
	6	93.758	66.706	32.003	75.659	≥ 70
	8	95.235	77.163	38.244	84.668	≥ 85
Test # 7	1	12.112	16.405	14.300	22.677	5-15
	2	56.537	30.672	20.753	42.709	25-45
	3	79.102	41.408	23.940	54.479	50-65
	4	91.463	51.803	25.314	65.156	≥ 70
	6	96.003	68.347	36.680	79.929	≥ 85

[a]Dissolution tests are as given in Table 1; [b]The values indicate average of cumulative % THP dissolved for six units; [c]% drug released specifications prescribed by USP 24, NF 19 through supplement 3 (Theophylline ER capsule formulation).

7.5 SIF (Test # 6), while the release rate was very slow in pH 1.2 SGF which was enhanced when the change-over medium of pH 6 phosphate buffer was used (Test # 1). Dissolution profile of this formulation was in close agreement with the dissolution limits for Test # 1 (Table 1 and 2).

On the other hand, in case of tablet formulation F2 the dissolution seemed to be independent of the dissolution conditions. It showed good dissolution behaviour in all the tested dissolution conditions (>80% of the labeled amount released within 9 h except in case of Test # 4/5) (fig. 2). The release pattern showed closeness with the USP specifications for Test # 1, 2 and 3 (Table 1 and 2).

Tablet formulation F3 showed condition-independent dissolution, but dissolution was poor as only about 48% of

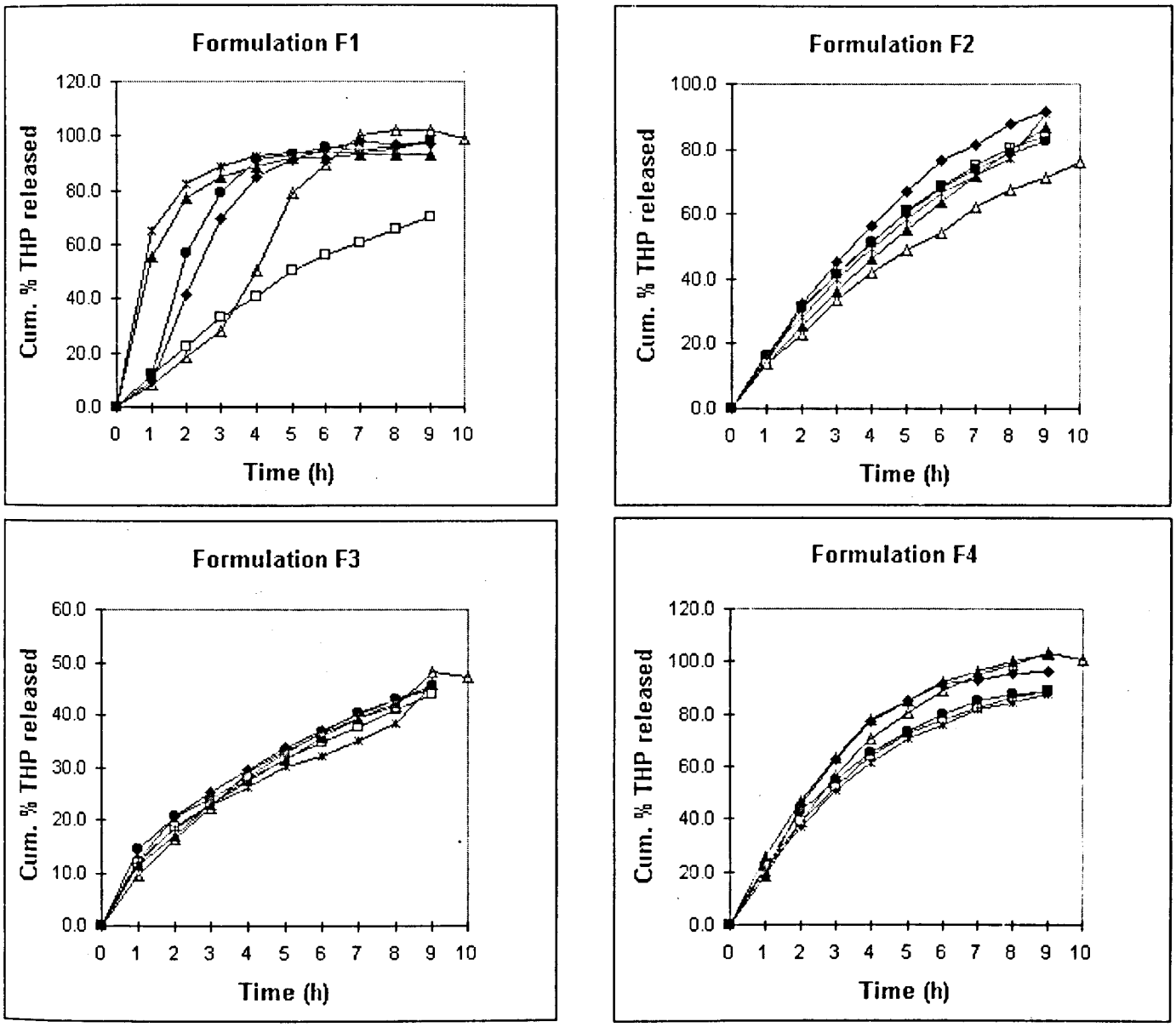


Fig. 2: Comparison of dissolution profiles of different MR formulations.

Comparison of mean *in vitro* release profiles for formulations F1, F2, F3 and F4 using different dissolution test conditions as described in Table 1 (n=6) [Test # 1, (-◆-); Test # 2, (-□-); Test # 3, (-▲-); Test # 4/5,(-Δ-); Test # 6, (-✶-); Test # 7, (-●-)].

the labeled amount of THP was released within 9 h (fig. 2). This formulation released THP very slowly as compared to other formulations and failed to comply with any of the dissolution test specifications (Table 1, 2).

In case of experimental formulation F4, cumulative % THP released *in vitro* appeared to be condition-independent and microspheres remained intact even after 9-10 h indicating that the drug may be released by diffusion mechanism. Almost 90% of the total drug content was released within 9-10 h (fig. 2). The cumulative % THP released at different time points was in very close agreement with the dissolution specifications for all the tests recommended by USP 24 for formulations to be administered every 12 h (i. e. Test # 1, 2, 4, 5, 6 and 7) (Table 1, 2).

Thus, out of four formulations studied, formulation F1 showed condition-dependent dissolution behavior, while formulations F2, F3 (tablets) and F4 (capsule) indicated condition-independent dissolution. Formulation F3, however, failed to comply with specifications of any of the studied dissolution conditions. Therefore, F3 was expected to exhibit only a compromised bioavailability profile.

Average plasma concentration (µg/ml) of THP versus time (h) profiles for all the four formulations (F1, F2, F3, and F4) are shown in fig. 3. The formulations showed that the C_{max} is reached at about 6 h after administration, confirming the modified release of THP. Various pharmacokinetic parameters obtained using noncompartmental analysis using WinNonlin-ProÔ are summarized in Table 3.

Formulation F1 showed C_{max} of 6.9 µg/ml and t_{max} was observed at 6 h. The values of AUC_{0-24} and K_{el} were comparable with that for formulation F2. It showed relatively more

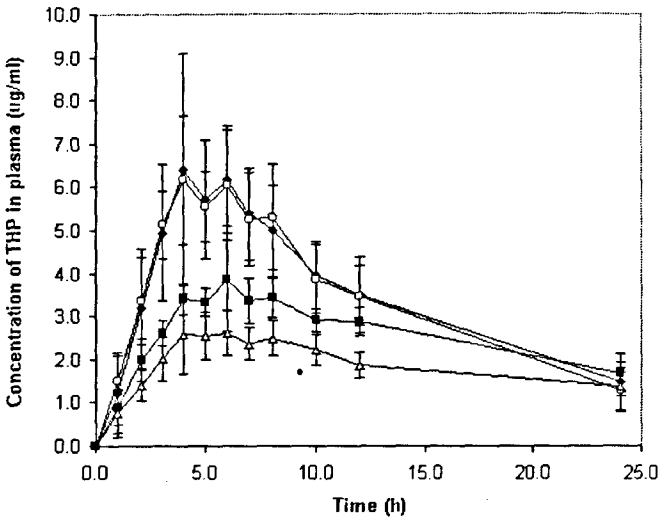


Fig. 3: *In vivo* profiles of different MR formulations of THP.

Average plasma concentration of THP (µg/ml) versus time (h) profiles for formulation F1 (-◆-), F2 (-O-), F3 (-Δ-) and F4 (-◘-) after administration into six healthy male volunteers.

variability within the subjects (SEM for C_{max}, t_{max} and AUC_{0-24} were 0.798 µg/ml, 0.543 h and 7.646 µg.h/ml, respectively) as compared to formulation F2 (Table 3). This may be because of dissolution condition-dependent (pH or ionic strength of medium) release behavior of F1.

Formulation F2, was found to perform in the similar fashion as did formulation F1. For this formulation, C_{max} was about 6.5 µg/ml, t_{max} was comparable (6 h) and K_{el} was nearing to the reported value (0.083 h^{-1})[19,20]. The value for AUC_{0-24} was found to be comparable with formulation F4. Further, this

TABLE 3: PHARMACOKINETIC PARAMETERS.

Pharmacokinetic parameter calculated	Formulation F1	Formulation F2	Formulation F3	Formulation F4
C_{max} (µg/ml)	6.949 ± 0.798	6.647 ± 0.591	2.955 ± 0.323	4.176 ± 00.413
t_{max} (h)	5.167 ± 0.543	5.667 ± 0.422	6.000 ± 0.683	5.333 ± 00.494
K_{el} (h^{-1})	0.071 ± 0.004	0.084 ± 0.007	0.046 ± 0.009	0.044 ± 00.008
$t_{1/2}$ (h)	9.937 ± 0.620	8.496 ± 0.630	17.621± 2.917	18.091± 02.739
AUC_{0-24} (µg.h/ml)	81.440 ± 7.646	80.595 ± 5.490	42.585 ± 3.051	60.676 ± 02.767
$AUC_{0-\infty}$ (µg.h/ml)	102.675 ± 9.458	96.808 ± 8.110	74.473 ± 8.805	107.736 ± 12.963

Note: Various pharmacokinetic parameters for different MR oral solid dosage forms determined by noncompartmental analysis using WinNonlin-Pro™. The values are given as mean ± SEM for data from six healthy volunteers.

formulation showed less inter-subject variability (SEM for C_{max}, t_{max} and AUC_{0-24} were 0.591 mg/ml, 0.422 h and 5.490 µg.h/ml, respectively). The values for C_{max}, AUC_{0-24} and t_{max} for F1 were in close agreement with corresponding parameters for formulation F2. Formulation F1 showed a relative bioavailability of 101.95% as compared to F2. Thus, formulation F1 was bioequivalent with formulation F2.

On the other hand, formulation F3, which had exhibited poor dissolution, showed as expected, very low C_{max} value (about 2.3 µg/ml), comparable t_{max} (about 6 h) and relatively lower K_{el} value. Even AUC_{0-24} was found to be as less as 42.585 µg.h/ml. This indicates poor bioavialablity of THP. It exhibited more inter-subject variation (SEM for C_{max}, t_{max} and AUC_{0-24} were 0.323 µg/ml, 0.683 h and 3.051 µg.h/ml, respectively), as compared to formulation F4.

C_{max} for microsphere formulation (F4) (~ 4.1 µg/ml) was comparable with the values reported in the literature[19,20] indicating its acceptable bioavilability, but was less as compared to F1(~ 6.9 µg/ml) and F2 (~ 6.6 µg/ml). t_{max} value was comparable with that of other study formulations, while K_{el} value was relatively less. This fornulation showed less AUC_{0-24} value as compared to that of formulation F1 (~ 81 µg.h/ml) and F2 (~ 80 µg.h/ml). Thus, it could not prove bioequivalence with either F1 or F2. It showed less inter-subject variability (SEM for C_{max}, t_{max} and AUC_{0-24} were 0.413 µg/ml, 0.494 h and 2.767 µg.h/ml, respectively). In case of formulations F3 and F4, K_{el} values were relatively less which may be due to underestimated elimination phase as sampling was carried out only upto 24 h after dose administration (fig. 3).

It was observed that out of the four formulations, F1 which exhibited condition-dependent THP release showed more inter-subject variation (with respect to major pharmacokinetic parameters, C_{max} and AUC_{0-24}) as compared to other formulations (F2, F3, F4) which showed condition-independent drug relesae.

Since neither the plasma concentration-time data after intravenous administration nor the data after administration of any fast releasing THP formulation was available, cumulative % THP absorbed after oral administration of the THP MR formulations was calculated using the Wagner-Nelson method[15].

TABLE 4: LINEAR REGRESSION ANALYSIS FOR *IN VITRO-IN VIVO* CORRELATION CURVES.

Formulation	Dissolution test condition [a] (IVIVC)						
		Test # 1	Test # 2	Test # 3	Test # 4/5	Test # 6	Test # 7
F1	Slope	1.0631	1.7338	1.1999	0.9081	1.1337	1.0759
	Intercept	3.0995	6.6705	-17.7095	24.4071	-16.4432	-1.5779
	R^2 [b]	0.9979	0.9811	0.9543	0.9555	0.9372	0.9942
F2	Slope	1.2139	1.2981	1.3521	1.3991	1.4034	1.3785
	Intercept	6.1701	8.3678	9.6100	12.1773	4.8949	4.0261
	R^2	0.9891	0.9886	0.9811	0.9739	0.9876	0.9895
F3	Slope	2.4122	2.4373	2.4022	2.3684	2.7605	2.4199
	Intercept	-0.6359	4.0279	3.0457	4.1522	-1.8626	-0.4850
	R^2	0.9905	0.9909	0.9879	0.9924	0.9891	0.9848
F4	Slope	1.0614	1.2341	1.0594	0.9980	1.2557	1.2848
	Intercept	0.1824	-0.6403	-1.9566	5.1298	-0.1267	1.2287
	R^2	0.9986	0.9958	0.9972	0.9933	0.9949	0.9956

Note: Linear regression equations for the *in vitro-in vivo* correlation curves for four different MR formulations of THP (F1, F2, F3 and F4) established using *in vitro* dissolution data generated using different dissolution test conditions. [a]Dissolution test conditions are as given in Table 1; [b]R^2 is the correlation coefficient for the linear regression line representing IVIVC.

The % THP absorbed values for each of the six volunteers after each treatment were subjected to ANOVA to evaluate significance of small population (n=6) for *in vivo* studies. It was found that 'F' values were less than the table values in case of all the four formulations demonstrating insignificant inter-subject variation after each treatment indicative of homogenous volunteer population.

To establish an IVIVC, % THP dissolved *in vitro* was plotted against % THP absorbed *in vivo* after oral administration over a period of 8 h (fig. 4). The values for slope, Y-intercept and R^2 for different regression lines obtained from these plots are given in Table 4.

In case of experimental formulation F4, the slope (slope=0.998 to 1.2848), intercept (intercept =-1.9566 to 5.1298) and correlation coefficient (R^2>0.99) data indicated that good correlation of *in vitro* dissolution and bioavailability is achieved independent of dissolution test conditions as per Table 1. However, ideal values for slope, intercept and

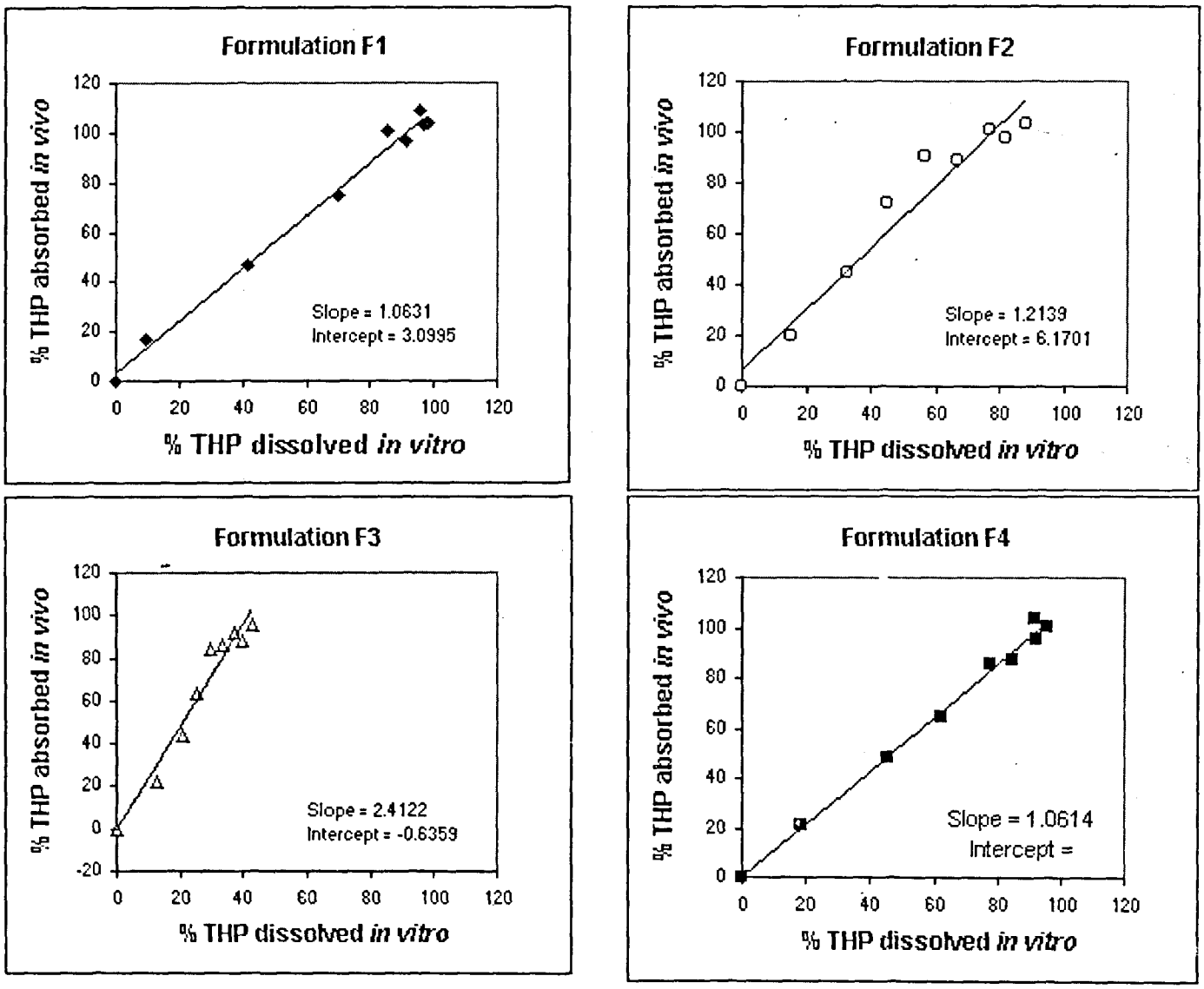


Fig. 4: *In vitro-in vivo* correlations for different MR formulations of THP.

***In vitro-in vivo* correlation for formulations F1, F2, F3 and F4 developed using mean *in vitro* dissolution data obtained by subjecting the formulations to the dissolution conditions of Test # 1 and the average plasma concentration of THP (µg/ml) versus time (h) data after administration into six healthy male volunteers.**

correlation coefficient, 1, 0 and =0.99, respectively, for a Level A correlation[18], were obtained when *in vitro* dissolution was carried out using pH 1.2 SGF (without pepsin) for 1 h followed by pH 6 phosphate buffer for the rest period as dissolution medium at 50 rpm (Test # 1) (fig. 4). Interestingly, all the four formulations performed well under this particular dissolution test condition with respect to their individual IVIVC (fig. 4). This may be because it mimics the *in vivo* environment of stomach and intestine in terms of pH, which the formualtion faces after oral administration. Out of all the test conditions, conditions of Test # 1 (Table 1) seemed to be best for all the four formulations when the values of slope, intercept and coefficient of correlation for corresponding IVIVCs were compared with those obtained using *in vitro* dissolution data under other dissolution conditions (Table 4). However, formulation F3 showed higher slope values (upto 2.4) indicative of lesser *in vitro* dissolution, but exhibited good correlation coefficient values (>0.99) for almost all the dissolution test conditions. In case of formulation F2, a good IVIVC (slope values ranging between 1.2 to 1.4 and correlation coefficient >0.99) was observed, but the values of intercept were higher (4.8949 to 12.1773). It probably indicates more bioavailability as compared to formulation F4 within 8 h and hence, higher C_{max} value. Finally, formulation F1 showed good correlation only when it was tested *in vitro* by using dissolution Test # 1. It demonstrated poor correlation when tested by other dissolution test conditions (R^2 values going down upto 0.9372), as its *in vitro* release is condition-dependent.

Thus, out of three market formulations, F1 and F2 showed acceptable dissolution and bioavailability. Interestingly, though formulations F1 and F2 are bioequivalent with overlapping plasma concentration versus time profiles upto 24 h (figs. 3 and 4) and comparable C_{max}, t_{max} and AUC_{0-24} values, their *in vitro* release behaviors are slightly different with respect to % cumulative THP released at different time points as per the USP specifications (Table 2). Though bioavailability of F4 is acceptable, is not bioequivalent with formulation F1 or F2. Thus, formulation F4 may need further modifications so as to achieve bioequivalence with formulation F1 or F2.

Dissolution studies performed using pH 1.2 SGF without pepsin (1 h) followed by pH 6 phosphate buffer (900 ml) (for rest period) with paddle (for tablets) or basket (for capsules) revolving at 50 rpm exhibited the best correlation between *in vitro* dissolution data and bioavailability of all the formulations studied (F1 and F4- capsules, F2 and F3- tablets, with different composition and process). These formulations showed either condition-dependent (F1) or condition-independent (F2, F3 and F4) *in vitro* dissolution behavior. Experimental formulation F4 showed good THP release profiles under different dissolution test conditions but could not achieve the bioavailability comparable with either formulation F1 or F2 and therefore, was not bioequivalent. Since, passing the dissolution test may not guarantee comparable bioavailability or bioequivalence, the correlation between dissolution and bioavailability needs to be demonstrated convincingly. Formulation F3 failed to comply with any of the dissolution specifications, showed very poor dissolution and also failed to ensure acceptable bioavailability of THP. Formulations F1 and F2 were found to be bioequivalent. Based on the above data it becomes clear that the dissolution conditions of Test # 1 can be employed in development of MR formulations of THP and also can serve as a quality control tool for such formulations.

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