Research Article

IN-VITRO AND SIMULATED *IN-VIVO* DISSOLUTION OF DIPYRIDAMOLE EXTENDED RELEASE CAPSULES

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ABSTRACT

In the present invention, an attempt made to evaluate the correlation between *in vitro* dissolution and *in vivo* dissolution, by incorporating enzymes to match the human body physiological conditions. Dipyridamole Extended release Capsules evaluated for dissolution in simulated fasting change over condition & Simulated Fed change over condition to synchronize the human body condition. In fasting change over condition, the dissolution is performed by using simulated dissolution media of pH 1.6 Fasted state simulated gastric fluid for 1 hr, followed by pH 6.0 Fasted state simulated intestinal fluid for 2 hrs followed by pH 7.2 Fasted state simulated intestinal fluid for 2 hrs, followed by pH 6.5 Fasted state simulated intestinal fluid for 4 hrs, followed by pH 6.8 Fasted state simulated intestinal fluid for 1 hr. In Fed change over condition, the dissolution is performed by using simulated dissolution media of pH 5.0 Fed state simulated gastric fluid for 1 hr, followed by pH 5.8 Fed state simulated intestinal fluid for 2 hrs, followed by pH 7.2 Fed state simulated intestinal fluid for 1 hr, followed by pH 6.5 Fed state simulated intestinal fluid for 5hrs followed by fed state simulated intestinal fluid pH 6.8 for 1 hr. The dissolution is performed for marketed product in the simulated condition, and found comparable. In addition to that the effect of surfactant in dissolution profiling is evaluated by using sodium lauryl sulfate as surfactant with different concentration levels, by suing 0.1M pH5.5 Phosphate buffer. No effect is observed up to 1.0% of sodium lauryl sulfate concentration in dissolution medium. Effect of agitation speed on dissolution evaluated and the agitation above 150 RPM is having effect on dissolution, due to centripetal force, so the dissolution values observed is lesser than 100-RPM values.

Keywords: *In vitro, In vivo*, Dipyridamole, Dissolution.

INTRODUCTION

Simulation of gastrointestinal conditions is essential to adequately predict the in vivo behavior of drug formulations. To reduce the size and number of human studies required to identify a drug product with appropriate performance in both the fed and fasted states, it is advantageous to be able to pre-screen formulations *in vitro*.[1-3] The choice of appropriate media for such in vitro tests is crucial to their ability to correctly forecast the food effect in pharmacokinetic studies

Orally administered drug products are the most dominant dosage forms. However, predicting oral drug absorption remains a challenge due to the variety of biopharmaceutical properties of the drug and drug products, as well as the complexity of gastrointestinal (GI) physiology. In healthy humans at fasted state, there are two important physiological factors impacting on drug dissolution and the subsequent absorption: 1) the hydrodynamics of GI tract; and 2) the components of GI fluids. The hydrodynamics of GI tract is intimately related to GI motility, which emcompasses gastric emptying, migrating motility complex (MMC), and the frequency and intensity of small intestine movement, while the critical GI fluid components are pH, bile salts and buffer species, volume, enzymes, osmolarity and calcium contents may be also important. For BCS II weak bases such as Dipyridamole (pKa: 5.7-6.4). The *in vivo* solubility and dissolution are more complex compared with the weak acids. Most of the dissolution related literature addresses the needs of QC, and only limited research has been invested to design BE dissolution methods. In establishing a meaningful BE dissolution methodology, two very important aspects must be considered: the hydrodynamic conditions along the GI tract and the complex contents of the GI fluids. The interplay between the GI hydrodynamics and GI fluids present the most challenging environment in designing a bio-relevant dissolution test[4-6]. In general, the surfactants play a major role on dissolution profile of extended release products, which will confirm the strength and integrity of coating. To confirm the integrity of coating, the dissolution is performed with different levels of sodium lauryl sulfate in 0.1M pH 5.5 phosphate buffer.

Multi particulate drug delivery systems are having more surface area for exposure in comparison to tablets. Hence, the percentage of coating required to control the dissolution of drug is high. To confirm the required percentage of coating, stress studies are carried out with different agitation speed in dissolution. To comply with current requirements of QBD (Quality by design), by regulatory bodies, such stress studies are required.

MATERIALS AND METHODS

Materials

Lecithin (VWR International Ltd, England),

Pepsin (VWR International Ltd, England),

Cholic acid (National Chemical, India, 99% purity),

Glyceryl monooleate (Triveni chemicals),

Sodium oleate (Triveni chemicals) and sodium taurocholate (Sigma–Aldrich Chemie GmbH, USA, 97% purity) were used. All reagents (potassium dihydrogen phosphate, Sodium dihydrogen phosphate, Sodium chloride, Sodium lauryl sulphate, Sodium acetate trihydrate, Sodium hydroxide, Hydrochloric acid, Maleic acid, glacial acetic acid, Orthophosphoric acid,) were of analytical grade (E Merck, India).

Methods

Preparation of Fasted state simulated fluid & Preparation of Fed state simulated fluid

Preparation of FaSSGF pH 1.6[2]

The composition, osmolarity and buffer strength of FaSSGF pH 1.6 is presented in table 1. FaSSGF pH 1.6 is prepared by dissolving 0.16 g Lecithin in 1.6 ml of Dichloromethane, 0.42 g of Sodium taurocholate in 5 liter of water. Add 1 g Pepsin and 20 g of NaCl. Heat the mixture to 40°C and make up the volume to 10 liters.

Table 1: Composition of the Medium to Simulate the Fasted – Fasted-State Simulated Gastric Fluid (FaSSGF)

Composition	
Sodium taurocholate (µM)	80
Lecithin (µM)	20
Pepsin (mg/mL)	0.1
Sodium chloride (mM)	34.2
Hydrochloric acid q.s. pH	1.6
Properties	
pH	1.6
Osmolality (mOsm/kg)	120.7 ± 2.5
Buffer capacity (mmol/L/pH)	–
Surface tension (mN/m)	42.6

Preparation of blank FaSSIF pH 6.0, pH 6.5, pH 6.8 & pH 7.2

The composition, osmolarity and buffer strength of FaSSIF pH 6.0, FaSSIF pH 6.5, FaSSIF pH 6.8 and FaSSIF pH 7.2 presented in table 2.

Table 2: Composition of the Medium to Simulate the Fasted – Fasted-State Simulated Intestinal Fluid pH 6.0, pH 6.5, pH 6.8 & pH 7.2

Composition				
Sodium taurocholate	3mM	3mM	3mM	3mM
Lecithin	0.75mM	0.75mM	0.75mM	0.75mM
Sodium dihydrogen phosphate	3.438g	3.438g	3.438g	3.438g
Sodium hydroxide q.s.pH	6.0	6.5	6.8	7.2
Sodium chloride	6.186g	6.186g	6.186g	6.186g
Deionised water qs	1L	1L	1L	1L
Properties				
pH	6.0	6.5	6.8	7.2
Osmolality (mOsm/kg)	270	270	270	270
Buffer capacity (mmol/L/pH)	12	12	12	12
Surface tension (mN/m)	54.3	54.3	54.3	54.3

Blank FaSSIF is prepared by dissolving 1.7g of sodium hydroxide pellets, 19.77g of sodium dihydrogen phosphate monohydrate and 30.93g of sodium chloride in 5L of purified water. Adjust the pH to exactly pH 6.0 or pH 6.5 or pH 6.8 or pH 7.2 using 1N Sodium hydroxide solution or 1N Hydrochloric acid solution.

Preparation of FaSSIF pH 6.0, pH 6.5, pH 6.8 & pH 7.2[7-9]

FaSSIF was prepared as follows: 3.3 g sodium taurocholate was dissolved in approximately 500 mL of the blank FaSSIF. The weight of this mixture was checked and noted ("weight 1"). Then 11.8 mL of a Methylene chloride solution containing 100 mg/mL lecithin (= 1.18 g lecithin, "weight 2") was added. This produced an emulsion (i.e., the resulting product was turbid). The Methylene chloride was then evaporated under vacuum using a Rotavap (type R-114, Buechi, Essen, Germany) at a temperature of about 40°C. About 10 min at 500 mbar followed by 30 min at about 50 mbar led to complete removal of the methylene chloride. The result was a clear, micellar solution having no perceptible odor of Methylene chloride. After cooling to room temperature, the weight of the solution was checked again. The water lost to evaporation was replaced with demineralized water to obtain a total weight corresponding to the sum of "weight 1" and "weight 2." Finally, the volume was brought to 2 L with blank FaSSIF.

Preparation of FeSSGF pH 5.0

The composition, osmolarity and buffer strength of FeSSGF pH 5.0 presented in table 3.

FeSSGF is prepared by dissolving 138.5 g NaCl, 40.04 g Sodium acetate in 5 L water and add 10 ml of Acetic acid and dilute to 10 liters with water.

Table 3: Composition of Fed State Simulated Gastric Fluid (FeSSGF)

Composition	
Sodium chloride (mM)	237.02 mM
Acetic acid	17.12mM
Sodium acetate	29.75mM
Milk / acetate buffer	1:1
Hydrochloric acid q.s. pH	5.0
Properties	
pH	5.0
Osmolality (mOsm/kg)	400
Buffer capacity (mmol/L/pH)	25

Preparation of blank FeSSIF pH 5.8, pH 6.5, pH 6.8 and pH 7.2

The composition, osmolarity and buffer strength of FeSSIF *pH 5.8, pH 6.5, pH 6.8 and pH 7.2* presented in table 4. Blank FeSSIF is prepared by dissolving 20.2g of sodium hydroxide pellets, 43.25g of glacial acetic acid and 59.37g of sodium chloride in 5L of purified water. Adjust the pH to exactly pH 5.8 or pH 6.5 using 1N sodium hydroxide solution or 1N Hydrochloric acid solution.

Table 4: Composition of the Medium to Simulate the Fed – Fed-State Simulated Intestinal Fluid pH 5.8, pH 6.5, pH 6.8 & pH 7.2 Composition of Fed State Simulated Intestinal Fluid (FeSSIF)

Composition				
Sodium taurocholate	10mM	10mM	10mM	10mM
Lecithin	2mM	2mM	2mM	2mM
Glyceryl monooleate	5mM	5mM	5mM	5mM
Sodium oleate	0.8mM	0.8mM	0.8mM	0.8mM
Maleic acid	55.02mM	55.02mM	55.02mM	55.02mM
Sodium chloride	125.5mM	125.5mM	125.5mM	125.5mM
Sodium hydroxide qs pH	5.8	6.5	6.8	7.2
Deionized water qs	1L	1L	1L	1L
Properties				
pH	5.8	6.5	6.8	7.2
Osmolality (mOsm/kg)	390 ± 10	390 ± 10	390 ± 10	390 ± 10
Buffer capacity (mmol/L/pH)	25	25	25	25

Preparation of FeSSIF pH 5.8 , pH 6.5, pH 6.8 and pH 7.2

FeSSIF was prepared by first dissolving 16.5 g sodium taurocholate in 500 mL of blank FeSSIF, checking and noting the weight ("weight 1"). Subsequently, 59.1 mL of a Methylene chloride solution containing 100 mg/mL lecithin (=5.91 g lecithin, "weight 2") was added, resulting in an emulsion. The Methylene chloride was then evaporated under the conditions described for FaSSIF until a clear, Micellar solution with no perceptible odor of Methylene chloride was obtained. After cooling to room temperature, the weight of the solution was checked again, and the water lost to evaporation was replaced with demineralized water to obtain a total weight corresponding to the sum of "weight 1" and "weight 2." Finally, the volume was brought to 2 L with blank FeSSIF.

Preparation of 0.1M pH 5.5 phosphate buffer with sodium lauryl sulphate buffer

0.1M pH 5.5 phosphate buffer is prepared by dissolving 13.61g of potassium dihydrogen phosphate in 1000ml of purified water, Adjust the pH to exactly pH 5.5 using 1N sodium hydroxide solution or 1N Hydrochloric acid solution.

Determination of Physicochemical Parameters

Various physicochemical parameters of the proposed dissolution media obtained as follows. The pH values measured using a digital pH meter (Lab India instruments, India). Osmolality determined by freezing point depression using an automatic Osmometer (Advance Instruments, USA). Buffer capacity was determined by titration with 1 M hydrochloric acid. Surface tension was measured using a stalagnometer.

Dissolution Test

The dissolution studies performed with USP Apparatus I (Varian Vankel, USA) employing 900 mL of dissolution media at 37 ± 0.5°C and a stirring rate of 100 rpm. A sample of approximately 10 mL was removed from each vessel using a cannula attached to a syringe (Becton Dickinson, USA) and was replaced immediately with approximately 10 mL of fresh medium at 37 ± 0.5°C. The samples were filtered through 0.45-µm filters and diluted with 0.1M HCl immediately.[1]

Dissolution Methods

The dissolution of Dipyridamole is performed by using UV-Visible spectrophotometer, comparison with the standard in the same medium having a known concentration of about 10 µg per mL, in 1-cm cells at the wavelength of maximum absorbance at about 282 nm. Calculate the quantity, in mg, of $C_{24}H_{40}N_8O_4$.

RESULTS AND DISCUSSION

The dissolution results in simulated fasting change over condition and simulated fed change over condition is comparable to marketed product. The results appended in table 5 & table 6 and graphical representation is in figure 1 & 2.

Table 5: Comparative dissolution profile of Test and Marketed product of Dipyridamole Extended Release capsules in simulated fasting change over condition

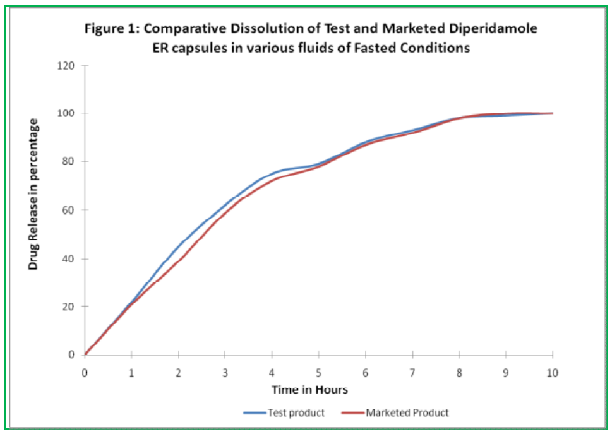
Product →		**Test product**	**Marketed product**
Batch number →		**F-9**	**702257**
Media and Time (h) ↓	**Cumulative time (h) ↓**	**Cumulative % drug release***	
0	0	0	0
pH 1.6 FaSSGF (1hr)	1	22	21
pH 6.0 FaSSIF (1hr)	2	45	39
pH 6.0 FaSSIF (2hrs)	3	62	59
pH 7.2 FaSSIF (1hr)	4	75	72
pH 7.2 FaSSIF (2hrs)	5	79	78
pH 6.5 FaSSIF (1hr)	6	88	87
pH 6.5 FaSSIF (2hrs)	7	93	92
pH 6.5 FaSSIF (3hrs)	8	98	98
pH 6.5 FaSSIF (4hrs)	9	99	100
pH 6.8FaSSIF (1hr)	10	100	100

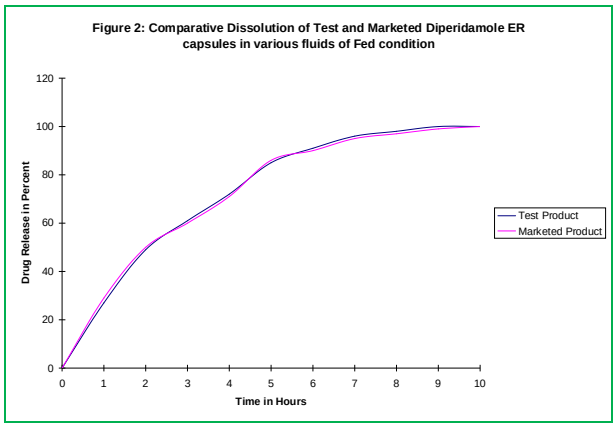
* Dissolution profiling in pH 1.6 FaSSGF for 1 hr followed by pH 6.0 FaSSIF for 2hrs followed by pH 7.2 FaSSIF for 2hrs followed pH 6.5 FaSSIF for 4hrs followed by pH 6.8 FaSSIF for 1hr, USP-I, 900ml, 100RPM

Table 6: Comparative dissolution profile of Test and Marketed product of Dipyridamole Extended Release capsules in simulated fed change over condition

Product →		**Test product**	**Marketed product**
Batch number →		**F-9**	**702257**
Media and Time (h) ↓	**Cumulative time (h) ↓**	**Cumulative % drug release***	
0	0	0	0
pH 5.0 FeSSGF (1hr)	1	27	29
pH 5.8 FeSSIF (1hr)	2	49	50
pH 5.8 FeSSIF (2hrs)	3	61	60
pH 7.2 FeSSIF (1hr)	4	72	71
pH 6.5 FeSSIF (1hr)	5	85	86
pH 6.5 FeSSIF (2hrs)	6	91	90
pH 6.5 FeSSIF (3hrs)	7	96	95
pH 6.5 FeSSIF (4hrs)	8	98	97
pH 6.5 FeSSIF (5hrs)	9	100	99
pH 6.8 FeSSIF (1hr)	10	100	100

* Dissolution in pH 5.0 FeSSGF for 1 hr followed by pH 5.8 FeSSIF for 2hrs, followed by pH 7.2 FeSSIF for 1hr, followed by FeSSIF pH 6.5 for 5hrs followed by pH 6.8 for 1 hr USP-I, 900ml, 100RPM.



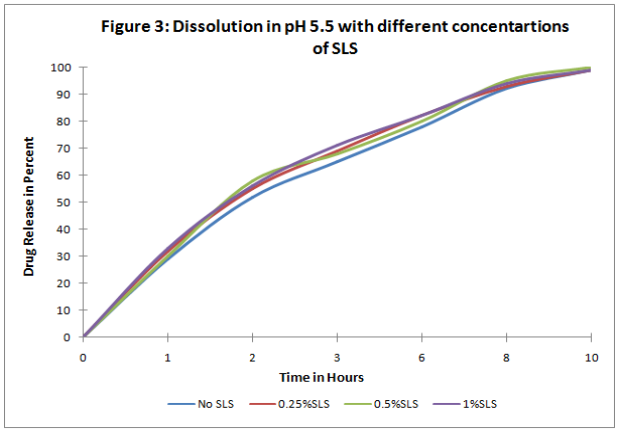


The dissolution results with different levels up to 1% w/w of sodium lauryl sulfate as surfactant is not showing difference in dissolution release. The results appended in table 7 and graphical representation in figure 3.

Table 7: Comparative dissolution profile of Dipyridamole Extended Release capsules with different concentration of surfactant (Sodium lauryl Sulphate) in dissolution media

Concentration of SLS →	without SLS	0.25% SLS	0.5% SLS	1% SLS
Time (hrs)	Cumulative % drug release			
0	0	0	0	0
1	29	32	30	33
2	52	55	58	56
3	65	69	68	71
6	78	82	80	82
8	92	93	95	94
10	99	99	100	99

* Dissolution in 0.1M pH 5.5 Phosphate buffer with different concentrations of sodium lauryl sulfate, USP-I, 900ml, 100RPM



Effect on dissolution profile is observed on different agitation speed up to 200 RPM. At 200 RPM, the dissolution profile is low due to centripetal force during dissolution in basket. Results are presented in table 8.

Table 8: Comparative dissolution profile of Dipyridamole Extended Release capsules with different Revolution per minute

RPM →	50	75	100	150	200
Time (hrs)	Cumulative % drug release				
0	0	0	0	0	0
1	25	28	29	30	22
2	48	51	52	52	35
3	63	64	65	64	48
6	76	78	78	76	62
8	90	90	92	95	82
10	98	98	99	100	88

* Dissolution in 0.1M pH 5.5 Phosphate buffer with different RPM, USP-I, 900ml.

CONCLUSION

The dissolution profile of test formulation is comparable to marketed formulation in simulated fasting change over condition and in simulated fed change over condition. The probability of being bioequivalence to marketed formulation is high.

The extended release pellet is not having effect on surfactant up to 1% w/w concentration of sodium lauryl sulfate in dissolution media. Hence, it can be concluded that, the extended release coating is not having impact by surfactant.

No effect on dissolution profile is observed on different agitation speed up to 150 RPM. Whereas, at 200 RPM, the dissolution profile is low due to centripetal force during dissolution in basket. Hence, it can be concluded, the extended release coating is having good strength and integrity.

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