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Research Article

DISCRIMINATORY POTENTIAL OF BIPHASIC MEDIUM OVER COMPENDIAL AND BIORELEVANT MEDIUM FOR ASSESSMENT OF DISSOLUTION BEHAVIOR OF TABLETS CONTAINING MELOXICAM NANOPARTICLES

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ABSTRACT

Objective: Dissolution test serves as a quality control tool for assessment of drug release from dosage form as well as a research tool to optimize new formulations. The existing guidelines by FDA, EMA, ICH, USP, etc., describe specifications for the dissolution of immediate release as well as modified release oral dosage form. However, none of them have discussed about the discriminatory potential of the medium to differentiate release profile of two or more products that are pharmaceutically equivalent. It is pertinent to add here that the pharmaceutical equivalents are not always bioequivalent. Hence, a discriminatory dissolution procedure is a must requirement to differentiate the release behavior of drug from a pharmaceutically equivalent product that contains different types and amount of excipient in the formulation. This also becomes more cumbersome when it is desirable for prediction of *in vivo* behavior of a drug when it is converted into a novel delivery system like nanoparticles. The reason could be the presence of excipients used to formulate drug nanoparticles into solid oral dosage form, may change the drug disintegration as well as dissolution behavior, which ultimately may lead to altered bioavailability.

Methods: In this study, the nanoparticles of meloxicam were prepared using wet media milling and the milled samples were dried using spray drier. The dried nanoparticles were converted into tablet dosage form by varying the type of diluent. To one batch lactose was used and another one was containing dicalcium phosphate (DCP). The assessment of release of meloxicam from these two batches was evaluated in various dissolution media.

Results: The study revealed that in all the cases the nanoparticulate tablets of Batch 1 have given increased dissolution profile as compared to marketed formulation (Muvera®), Batch 2 and controlled tablets of meloxicam. This proved that the excipients also play a major role in the release behavior of drug otherwise if it was not so, the nanoparticulate tablets of Batch 1 and Batch 2 would have given the same dissolution profile in all the tried media. Batch 1 containing lactose with a higher surface area provided more and rapid wetting of the drug by the dissolution media compared to Batch 2 that contained DCP as a major diluent.

Conclusion: Among all the dissolution media tried to evaluate the discriminatory power and simulation with a biorelevant medium, the biphasic medium of pH 1.8, 4.8 and 6.8 has promised to simulate with biorelevant media. However, the medium of pH 6.8 has shown the best dissolution profile.

Keywords: Solubility, Compendial media, Biphasic media, Dissolution, Meloxicam.

INTRODUCTION

Meloxicam is an antirheumatoid drug which falls in the category of non-steroidal anti-inflammatory drugs. "Previous studies on meloxicam pharmacokinetics shown that after oral administration, it has slow absorption with T_{max} that is longer than 5 hrs [1-5]. In comparison, intramuscular injection of meloxicam reached the maximum plasma concentration (C_{max}) within 1.5 hrs of administration and 90% of the C_{max} within 30-50 minutes over the dose range of 5-30 mg in humans [6,7]. Hence, intramuscular administration of meloxicam could shorten the onset of action since rapid pain relief is required in the case of acute and painful exacerbations of rheumatoid arthritis. However, due to the potential local tissue irritation and necrosis, intramuscular administration of meloxicam is not recommended for the chronic use and should be switched to oral formulation as soon as the rapid onset of action is achieved [7]. Hence, the development of an oral formulation of meloxicam with faster onset of action while maintaining the prolonged exposure could be a very good alternative." It belongs to BCS Class II and possesses poor solubility and thereby dissolution rate limited oral bioavailability. The details about physicochemical properties of meloxicam are shown in Table 1.

In last one decade, various approaches have been reported to improve the dissolution rate of meloxicam. Some of them include solubilization in surfactant solutions, the use of cosolvents, pH adjusted solutions, emulsions, liposomes, complexation with cyclodextrins, and solid dispersions [10-16].

However, above-mentioned techniques have some or the other limitations, like difficulty to scale up, clinical toxicity or stability, etc.

Nanosuspensions, on the other hand, have proven to be the cornerstone approach to overcome dissolution rate limited bioavailability of poorly soluble drugs [17].

There are several reports to overcome stability related problems, wherein nanosuspension have been prepared and been successful to get marketed [18]. Despite this the physiochemical stability related challenges such as sediments and Ostwald's ripening cannot be ignored. To overcome such problems, solidification of nanosuspensions through spray drying or lyophilization and the conversion into tablets, or capsules is now greatly practiced. Among, various oral dosage form systems, tablets are considered as the most common and convenient route due to their acceptable patient compliance, exposure of drugs to large surface area, rich blood supply, prolonged drug retention, advantage of scale up, and commercialization [18].

Dissolution test is utilized as either a research tool for optimizing new formulations or a quality control test to monitor the uniformity and

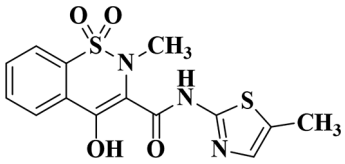
reproducibility of production batches. In biological systems, drug dissolution is an important attribute before systemic absorption [19]. The dissolution test also reflects significant differences in bioavailability arising from differences in dissolution [20] and discriminate formulation factors such as polymers, particle surface area, and physical and chemical characteristics of the drug [21,22]. When dissolution testing is used to forecast the *in vivo* performance of a drug, it is critical that the *in vitro* test should mimic the *in vivo* conditions as closely as possible. The nature of the dissolution medium affects the dissolution rate [23,24]. It is pertinent to add here that a medium that could be able to discriminate the release behavior of drug, either in its conventional or novel form due to the type of excipients used in the formulation could help the formulation scientists to overcome the challenges related to batch to batch variations as well as quality of dosage form prepared, which are pharmaceutically equivalent. Such attempts could also provide some regulatory considerations related to bioequivalence studies of products. In the present study was aimed to evaluate the potential of biphasic media to predict *in vivo* performance of meloxicam nanoparticulated tablets.

EXPERIMENTAL

Materials

Meloxicam was procured from Jackson Pharmaceuticals, Amritsar, India. Marketed formulation of meloxicam "Muvera®" was purchased from local chemist. Phospholipid (Egg lecithin) was gifted by Lipoid, Germany. Sodium lauryl sulfate (SLS) and hydroxypropyl methyl cellulose (HPMC) were purchased from Loba Chemie, India. Lactose monohydrate, dicalcium phosphate (DCP) and magnesium stearate were purchased from Qualikems fine chemical Pvt. Ltd., India. Sodium dihydrogen phosphate, potassium dihydrogen orthophosphate, boric acid, sodium hydroxide, sodium chloride, microcrystalline cellulose, octanol, and tritonX100 were purchased from Central Drug laboratories, India. Sodium taurocholate was purchased from Sigma-Aldrich life sciences, India. Crosspovidone (Kollidon CL) was purchased from BASF, India. Milk was purchased from Heritage, India. Triple distilled water was used throughout the study.

Table 1: Description of meloxicam [8,9]

Parameters	Description
Molecular structure	
Molecular weight	351.401
Chemical formula	$C_{14}H_{13}N_3O_4S_2$
Melting point	254°C
Solubility in water	7.15 mg/L (0.154 mg/mL)
Solubility in different pH based aqueous buffers	Meloxicam has pH dependent solubility. It is an example of acidic NSAID. The aqueous solubility of acidic NSAIDs is pH dependent. Decrease in pH leads to an increase in the ratio of non-ionized to ionized drug, combined with decrease in solubility with decreasing pH and vice versa with increase in pH
Log P (water/octanol distribution coefficient)	3.43
$CaCO_2$ permeability	−4.71
pKa (Strongest acidic)	4.47
pKa (Strongest basic)	0.47

NSAIDs: Non-steroidal anti-inflammatory drugs

Preparation of nanosuspension

The nanosuspension of meloxicam was prepared by the method reported in our recent published work [25]. The known amount of meloxicam was first dispersed in the dispersion medium containing known amount of HPMC and SLS and mixed in a mechanical mixer at 500 rpm. Afterward the suspension was milled using bead mill (Model: Lab Star 1, Netzsch Mill, Germany) for particle size reduction. Prepared nanosuspension was dried using spray drier (Spray Mate, Jay Instruments Pvt. Ltd. India). A batch of 250 g meloxicam nanosuspension was poured in spray dryer to make it into fine drug nanoparticles. Dried nanoparticles were collected at the end of the cyclone separator.

Characterization of nanosuspension

Prepared nanosuspension was characterized for particle size, zeta potential, % drug loading, and stability as per the procedure discussed in our previous report [25]

Compression of nanoparticles and pure drug to tablet dosage form

Spray dried nanoparticles were converted into tablets by varying the type of diluents. Batch 1 was containing lactose monohydrate as diluent, whereas Batch 2 was containing DCP as diluent (Table 2). Lactose is water soluble excipient, forms more microcavities in the polymer in polymer matrices. Hence, help in better swelling and disintegration and dissolution. Whereas DCP is water-insoluble excipient which causes less prominent swelling, erosion, and drug release sustaining properties in matrices. On the other hand, DCP provides a better compaction as that of lactose [26]. Hence, the impact of this excipient in release of meloxicam nanoparticles in various pH dependent media is worth exploring.

To compare their dissolution profile with micronized form of meloxicam, a controlled batch was also prepared, wherein unmilled meloxicam was kept as API.

All of the materials were passed through sieve No. 60 before use and the accurately weighed amounts of ingredients were thoroughly mixed and compressed into 100 mg tablets using multi punch machine (Trover Pharm, India) of 8 mm flat punch and die set.

In vitro disintegration time

The disintegration test has been carried out for prepared tablets (Batches 1 and 2), Muvera® (marketed formulation of meloxicam) and

Table 2: Batch composition of various batches of tablets containing meloxicam nanoparticles

Batch	Meloxicam SD (mg/tablet)	Lactose monohydrate (Pharmatose® - DCL 11) (mg/tablet)	Dicalcium phosphate (mg/tablet)	Microcrystalline cellulose (Avicel® pH 112) (mg/tablet)	Crospovidone (Kollidon® CL) (mg/tablet)	Colloidal silicon dioxide (Aerosil® 200) (mg/tablet)	Magnesium stearate (Panreac®) (mg/tablet)
Batch 1	11.55 (containing meloxicam equvalent to 7.5 mg)	66.52	-	16.63	3.8	0.5	1
Batch 2	11.55 (containing meloxicam equvalent to 7.5 mg)	-	66.52	16.63	3.8	0.5	1
Control Batch 3	7.5	68	-	18	5	0.5	1

controlled Batch in different media which were used for dissolution studies.

The test was carried out on six tablets using tablet disintegration tester (DT1000, Lab India). For the study, 900 ml of phosphate buffer of pH 6.8 was used. The temperature of the medium was maintained at 37°C±0.5°C. "The time taken for complete disintegration of the tablet with no palpable mass remaining in the apparatus was measured in seconds" [27].

***In vitro* dissolution studies**

The dissolution study was performed for Batches 1 and 2 containing meloxicam nanoparticles (equivalent to 7.5 mg), marketed formulation (Muvera®), and controlled batch, in dissolution tester USP II apparatus (DS8000, LAB INDIA). The study was conducted in three different types of media, which includes, Compendial pH buffer media, biorelevant media and biphasic media at temperature 37°C±0.5°C and 50 rpm agitation speed.

At specified time intervals of 5, 10, 15, 20, 30, 45, 60, 75 and 90 minutes, "5 ml of dissolution medium was withdrawn and replaced with an equal volume of medium to maintain a constant total volume" [28]. Samples were filtered through a 0.45 µm membrane filter, and the samples were analyzed at 359 nm using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan).

Composition of dissolution media

All the compendial buffers were prepared as per USP 31 NF 27 [29]. The composition of different dissolution media are as follows:

HCL (0.2 N) buffer solution of pH 1.8

Potassium chloride (3.7 g) and concentrated hydrochloric acid (7 ml) were added to 1000 ml volumetric flask and volume was made up to 1000 ml using distilled water. The pH of the buffer was adjusted to 1.8 using 0.1 N HCL or 0.1 N NaOH. Phthalate (0.2 N) buffer solution of pH 4.8.

Potassium hydrogen phthalate (10.4 g) and NaOH (6.6 g) were added to 1000 ml volumetric flask and volume was made up to 1000 ml using distilled water. The pH of solution was adjusted to 4.8.

Phthalate buffer (0.2 N) solution of pH 5.6

Potassium hydrogen phthalate (10.4 g) and NaOH (7.1 g) were added to 1000 ml volumetric flask and volume was made up using distilled water. The pH of solution was adjusted to 5.6.

Phosphate buffer (0.2 N) solution of pH 6.8

Potassium dihydrogen orthophosphate (6.8 g) and NaOH (9.0 g) were added to 1000 ml volumetric flask and volume was made up to 1000 ml using distilled water. The pH of solution was adjusted to 6.8.

Phosphate buffer (0.2 N) solution of pH 7.4

Potassium dihydrogen orthophosphate (6.8 g) and NaOH (16.9 g) were added to 1000 ml volumetric flask and volume was made up using distilled water. The pH of solution was adjusted to 7.4.

Borate buffer (0.2 N) solution of pH 9.6

Boric acid (3.1 g), 3.7 g of potassium chloride, and 14.7 g of NaOH were added to 1000 ml volumetric flask and volume was made up using distilled water. Solution pH was adjusted to 9.6.

Fasted state simulated gastric fluid (FaSSGF) [29-35]

This media was prepared mixing 2 g of sodium chloride, 3 g of hydrochloric acid, 1 g of tritonX 100-1000 ml volumetric flask and volume was made up using distilled water. The pH of solution was adjusted to 1.8. The composition of FaSSGF is shown in Table 3.

Fasted state simulated intestinal fluid (FaSSIF) [29-35]

This medium was prepared in two steps, wherein first step describes about the preparation of blank FaSSIF and the second step describes the preparation of standard solution of FaSSIF. Blank was prepared by adding 3.48 g sodium dihydrogen orthophosphate, 6.1 g of sodium chloride, 0.34 g of sodium hydroxide to 1000 mL volumetric flask and volume was made up using distilled water. Final FaSSIF was prepared by adding 1.65 g of sodium taurocholate and 0.519 g of lecithin to 1000 ml volumetric flask and volume was made up using blank FaSSIF. pH of solution was adjusted to 6.5. The composition of blank FaSSIF and FaSSIF is shown in Tables 4 and 5.

Fed state simulated intestinal fluid (FeSSIF) [29-35]

This medium was prepared in two steps, wherein first step describes about the preparation of blank FeSSIF and the second step describes the preparation of standard solution of FeSSIF. Blank FeSSIF was prepared by adding 8.65 g of glacial acetic acid, 11.87 g of sodium chloride, 4.04 g of sodium hydroxide to 1000 ml volumetric flask and volume was made up using distilled water. The final solution was prepared by adding

Table 3: Composition of fasted state simulated gastric fluid [29-35]

Material	Quantity (g)
Sodium chloride	2
Hydrochloric acid	3
TritonX 100	1
Distilled water qs	1 L

qs: Quantity sufficient

Table 4: Composition of blank fasted state simulated intestinal fluid [29-35]

Material	Quantity (g)
Sodium dihydrogen orthophosphate	3.40
Sodium chloride	6.20
Sodium hydroxide	0.34
Distilled water qs	1 L

qs: Quantity sufficient

Table 5: Composition of FaSSIF [29-36]

Material	Quantity (g)
Sodium taurocholate	1.65
Lecithin	0.59
Blank FaSSIF qs	1 L

qs: Quantity sufficient, FaSSIF: Fasted state simulated intestinal fluid

Table 6: Composition of blank FeSSIF [35]

Material	Quantity (g)
Glacial acetic acid	8.65
Sodium chloride	11.87
Sodium hydroxide	4.04
Distilled water qs	1 L

qs: Quantity sufficient, FeSSIF: Fed state simulated intestinal fluid

Table 7: Composition of FeSSIF [35]

Material	Quantity (g)
Sodium taurocholate	8.25
Lecithin	2.96
Blank FeSSIF qs	1 L

qs: Quantity sufficient, FeSSIF: Fed state simulated intestinal fluid

8.25 g of sodium taurocholate, 2.96 g of lecithin to 1000 mL volumetric flask and volume was made up using blank FeSSIF. pH of solution was adjusted to 5.0. The composition of blank FeSSIF and FeSSIF is shown in Tables 6 and 7.

Biphasic dissolution medium

This media contains two phases; organic phase and an aqueous phase. Octanol was taken as organic phase and buffer solution was taken as the aqueous phase. The biphasic media of different pH 1.8, 4.8, 6.8, 7.4 were prepared by adding 100 ml of octanol to 800 ml of aqueous buffer, whose preparation was discussed in previous sections.

Composition of milk medium

Simulated gastric fluid (SGF) medium containing milk was prepared using equal parts of milk and SGF pH 1.2. The final pH was taken to 3.0 with either 0.1 M HCl or 0.1 M NaOH. The SGF composition was already discussed in earlier section. Different milk media were prepared which are (a) whole fat milk 50%, (b) semi skimmed milk, and (c) skimmed milk.

Statistical analysis of data

The *in vitro* release profiles of meloxicam nanosuspension were compared using model independent analysis for calculation of similarity factor as defined by the following equation.

$$f_2 = 50\log\left\{\left[1+\frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)\right]^{-0.5}\times 100\right\}$$

Here, n indicates number of time points at which % drug dissolved was determined, R_t is the % drug dissolved of one formulation at a given time point and T indicates the % drug dissolved of the formulation to be compared at the same time point [37,38].

RESULTS AND DISCUSSION

Characterization of meloxicam nanoparticles [25]

Mean particle size distribution d (50) of prepared nanoparticles of meloxicam was found to be 265 nm with 65.21% drug loading and zeta potential of −25.26 mV. The dissolution profile of fresh nanosuspension and aged nanosuspension (Kept for stability studies) showed that the aged nanosuspension appeared to have lower dissolution rate than the fresh nanosuspension in the graph; however, a similarity factor (F2) of 53.25 indicated that the two release profiles were acceptably similar [25].

Solubility studies of prepared nanoparticles and API in different dissolution media

Table 8 represents the saturation solubility of meloxicam nanoparticles and pure API in in 250 mL of media, respectively, in different dissolution media, which includes, compendial pH 1.8, 4.8, 5.6, 6.8, 7.4, 9.6, FaSSGF, FaSSIF, FeSSIF, biphasic media of pH 1.8, 4.8, 6.8, 7.4. Pure meloxicam showed maximum solubility of 4.147±0.95 µg/ml in the biphasic medium at pH 7.4. Meloxicam nanoparticles have showed a profound increase in solubility, with a maximum solubility of 46.82±0.23 µg/ml in the biphasic medium at pH 7.4 with a value of Cs/Cd 4.071. A value above 3 represents a very

Table 8: Saturation solubility (mean±SD) of meloxicam API and nanoparticles in different dissolution media

Dissolution media	Mean±SD (n=3*)					
	Pure API (amount of drug dissolved) in µg/mL	Sink condition (Cs/Cd)	Pure API (percentage solubility) in %	Nanoparticles (amount of drug dissolved) in µg/mL)	Sink condition (Cs/Cd)	Nanoparticles (percentage drug dissolved in %)
pH buffer 1.8	0.001±0.0001	-	0.002±0.001	8.43±0.18	0.73±0.06	16.86±1.18
pH buffer 4.8	0.004±0.002	-	0.008±0.003	11.26±0.56	0.98±0.67	22.52±1.24
pH buffer 5.6	0.16±0.004	0.014±0.001	0.32±0.011	14.94±1.44	1.30±0.93	29.88±2.11
pH buffer 6.8	0.03±0.005	0.002±0.001	0.06±0.024	26.71±1.05	2.32±0.17	53.42±2.73
pH buffer 7.4	0.05±0.008	0.004±0.001	0.10±0.004	37.93±1.34	3.30±0.72	75.86±3.16
pH buffer 9.6	0.28±0.002	0.025±0.002	0.51±0.05	44.02±2.19	3.83±0.43	88.40±2.17
FaSSGF	0.76±0.014	0.07±0.006	1.53±0.21	12.36±0.35	1.07±0.32	24.72±1.94
FaSSIF	1.21±0.003	0.11±0.018	2.43±0.19	28.64±0.83	2.50±0.76	57.28±1.11
FeSSIF	0.97±0.013	0.08±0.009	1.94±0.32	19.27±1.93	1.68±0.22	38.54±0.87
Biphasic media pH 1.8	2.46±0.026	0.21±0.023	4.93±0.22	29.69±2.85	2.58±0.71	59.38±1.45
Biphasic media pH 4.8	2.63±0.018	0.23±0.013	5.27±0.48	38.30±1.91	3.33±0.88	76.60±1.92
Biphasic media pH 6.8	3.81±0.014	0.33±0.009	7.62±0.32	43.17±1.02	3.75±0.72	86.34±0.93
Biphasic media pH 7.4	4.15±0.95	0.36±0.010	8.29±0.92	46.82±0.23	4.07±1.14	93.64±2.12

n=3*: Number of replicates of study=3. FaSSGF: Fasted state simulated gastric fluid, FaSSIF: Fasted state simulated intestinal fluid, FeSSIF: Fed state simulated intestinal fluid, SD: Standard deviation

Table 9: Disintegration results (mean±SD) for prepared tablets

Media used	Mean±SD (n=3*)			
	Marketed (Muvera®) (min)	Batch 1 (min)	Batch 2 (min)	Controlled batch (Batch 3) (min)
Buffer pH 1.8	2.36±0.21	0.41±0.04	0.27±0.021	2.24±048
Buffer pH 4.8	2.17±0.41	0.47±0.08	0.28±0.019	2.21±0.23
Buffer pH 5.6	2.25±0.89	0.45±0.10	0.21±0.054	2.16±0.16
Buffer pH 6.8	2.11±1.12	0.37±0.09	0.31±0.029	2.27±0.37
Buffer pH 7.4	2.17±0.98	0.39±0.12	0.29±0.087	2.28±0.98
Buffer pH 9.6	1.58±0.21	0.34±0.076	0.28±0.042	2.26±0.016
FaSGF – 1.8	2.34±0.32	0.42±0.052	0.30±0.066	2.20±0.32
Fassif – 6.5	2.13±0.24	0.36±0.018	0.32±0.042	2.34±0.65
FeSSIF – 5.0	2.56±0.34	0.45±0.022	0.35±0.078	2.37±0.31
Biphasic media pH 1.8	2.35±1.12	0.41±0.067	0.27±0.092	2.21±0.39
Biphasic media pH 4.8	2.17±0.21	0.45±0.054	0.28±0.040	2.26±0.48
Biphasic media pH 6.8	2.24±0.37	0.32±0.087	0.27±0.034	2.27±0.64
Biphasic media pH 7.4	2.12±0.65	0.35±0.076	0.31±0.028	2.28±0.27

n=3* : Number of replicates of study=3. FaSSGF: Fasted state simulated gastric fluid, FaSSIF: Fasted state simulated intestinal fluid, FeSSIF: Fed state simulated intestinal fluid, SD: Standard deviation

good sink condition [39]. This may be due to increase in surface area and wettability.

***In vitro* disintegration time**

Nanoparticulated tablets showed less disintegration time when compared to marketed and controlled. However, all the tablets have passed pharmacopoeial limits (less than 5 minutes). The results are shown in Table 9.

***In vitro* dissolution testing**

Dissolution in compendial medium

To evaluate the discriminatory power and drug release potential, the dissolution studies were carried out in conventional media of pH 1.8, 4.8, 5.6, 6.8, 7.4 and 9.6 buffers, respectively, using the marketed formulation (Muvera®), controlled formulation, Batches 1 and 2 of nanoparticulated meloxicam tablets.

In Fig. 1, it was observed that the Batch 2 and controlled batch have given a poor drug release as compared to Batch 1 and Muvera®. This clearly shows that the effect of DCP on drug release of Batch 2 and lactose monohydrate on drug release of Batch 1. The decrease in drug release profile of Batch 2 was mainly due to the presence of DCP that forms stagnant layer over the meloxicam nanoparticles, and thus, the dissolution medium could not sufficiently wet the meloxicam nanoparticles. However, in Batch 1, lactose monohydrate which was almost amorphous in nature and has larger surface area provided immediate wetting to the meloxicam nanoparticles after disintegration, and thus, enhanced its drug release.

It was observed that no sufficient sink conditions have been achieved with any of the formulations at pH 1.8. The maximum drug release found with Batch 1 was 12.45%, Batch 2 with 6.72%, Muvera® with 9.63%, and controlled batch with 3.9%, respectively, in 75 minutes, which was much lesser as far as the limit of quantification is concerned for an immediate release dosage form (i.e., 75% in 15 minutes) [28].

Another experiment carried out at pH 4.8 and 5.6 buffer also showed the same results with little increase in dissolution profile (i.e., Batch 1 with 42.7%, Batch 2 with 10.51%, Muvera® with 46.5%, and controlled batch with 6% at pH 5.6). The results are shown in Fig. 2 and Batch 1 with 52.4%, Batch 2 with 14.6%, Muvera® with 46.4%, and controlled with 6% at pH 4.8 as shown in Fig. 3. This revealed that at pH 4.8 four folds increase in dissolution profile was observed with Batch 1, two folds with Batch 2,

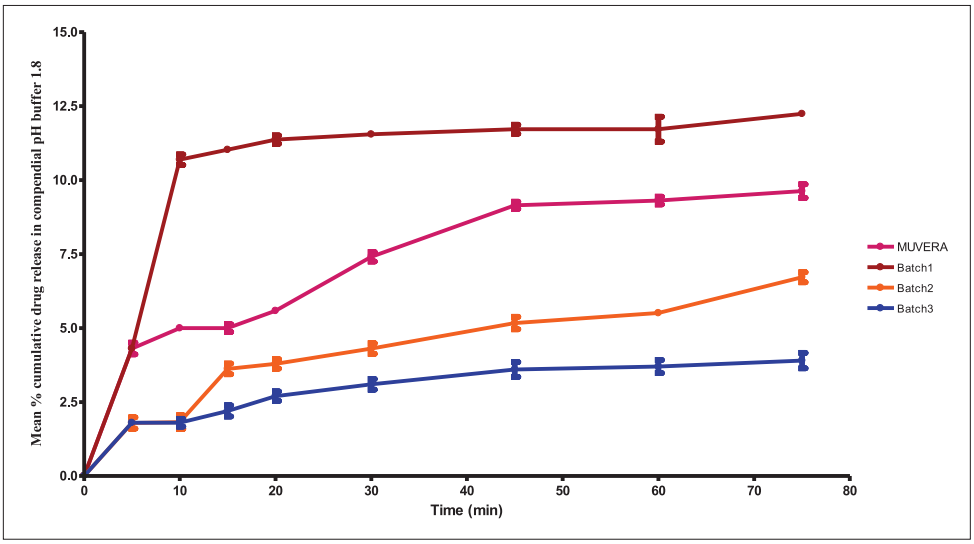


Fig. 1: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in compendial pH buffer 1.8

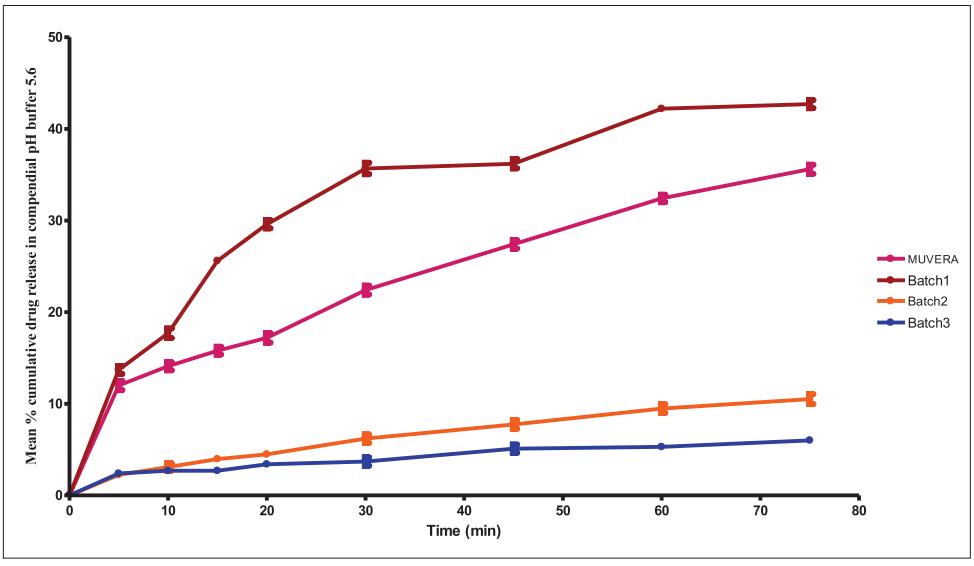


Fig. 2: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in compendial pH buffer 5.6

five folds increase with Muvera®, and two folds increase with controlled batch as compared to drug release at pH 1.8. At pH 5.6, four folds increase in drug release was found with Batch 1, 1.5 folds increase with Batch 2, four folds increase with Muvera® and two folds increase with controlled batch as compared to pH 1.8. However, the drug release was still not achieved with this dissolution media as per compendial requirements.

From Fig. 4, revealed 68.4 % drug release with Batch 1, 35.8% with Batch 2, 62.9% with Muvera®, and 37.2% with controlled at pH 6.8. This showed that a 5.5 folds increase in drug release with Batch 1, 8.5 folds increase with Batch 2, 7 folds increase with Muvera®, and 12 folds increase with controlled as compared to pH 1.8. Fig. 5 shows the drug release in pH 7.4, as Muvera® with 64 %, Batch 1 with 65.7%, Batch 2 with 45%, and Controlled batch with 39.31%. However, still the compendial requirements have not been achieved. When dissolution studies were carried out at pH 9.6, 71.3% drug release was observed with Batch 1, 68.1% drug release with Batch 2, 68.79% with Muvera® and 68.13 % with controlled one (Fig. 6). Here, six folds increase was observed with Batch 1, 5.5 folds increase with Batch 2, 5.5 folds increase with Muvera® and 24 folds increase controlled batch as compared to pH 1.8. In this case, Batch 1 has fulfilled compendial requirements; however, still the other formulations have not shown a proper drug release as per compendial requirements. This proved that the drug is not only having pH dependent solubility profile but it is also considered as poorly soluble drug because even 900 ml of dissolution media at pH 9.6 has not shown 100% drug release in 75 minutes and thus no proper sink conditions has been achieved for meloxicam. The US-FDA guideline do not allow the use of pH 9.6 buffer for routine quality control analysis of drug because they are not considered as physiologically relevant medium (i.e. not in range of 1.8-7.5). Furthermore, pH above 8.0 causes column degradation since nowadays most of the dissolution apparatus are coupled with autosampler high performance liquid chromatography [40].

All these problems proved that the normal compendial media cannot surrogate the *in vivo* performance of meloxicam. Hence, it warranted to develop a medium which should be rich with phospholipids and bile salts to mimic the *in vivo* physiological conditions. Hence, an attempt has been made to use biorelevant media to evaluate the drug release profile of above said four formulations. In view of this, six different biorelevant media have been prepared which are FaSSGF (pH 1.8), FaSSIF (pH 6.5), FeSSIF (pH 5.0), SGF containing whole fat milk (pH 3.0), SGF containing semi-skimmed milk (pH 3.0), and SGF containing skimmed milk (pH 3.0). The compositions of these media are already discussed above.

The dissolution studies carried out in FaSSGF (pH 1.8) (Fig. 7) showed that 57.41% drug release from Batch 1, 47.5% drug release

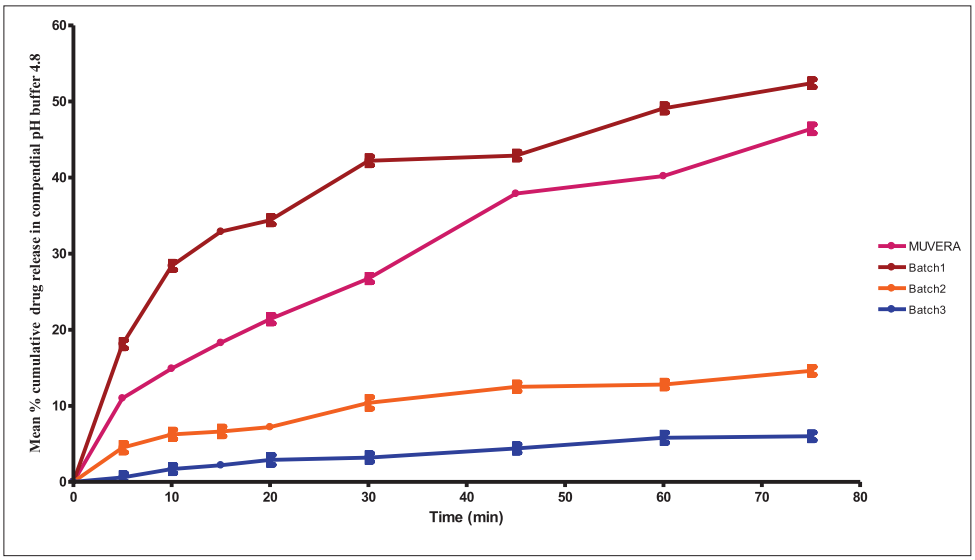


Fig. 3: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in compendial pH buffer 4.8

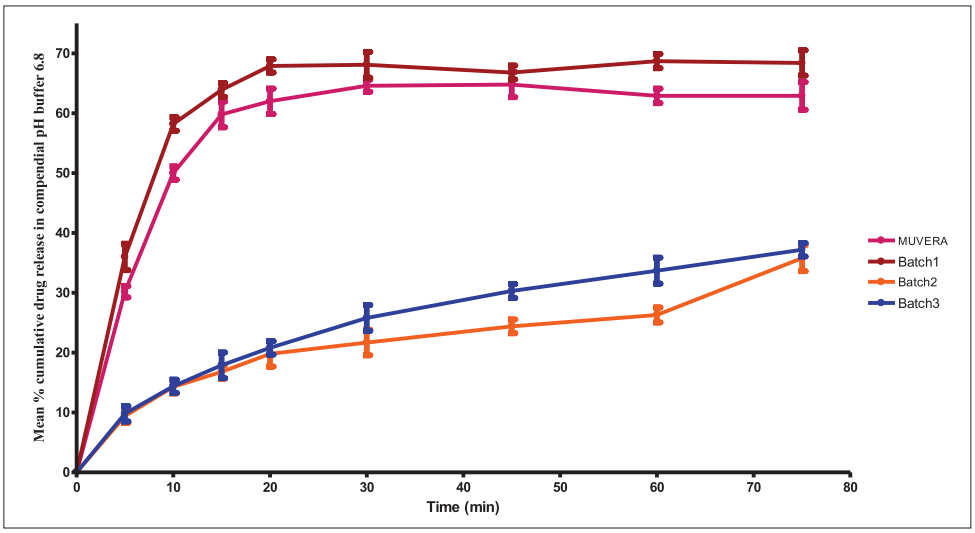


Fig. 4: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in compendial pH buffer 6.8

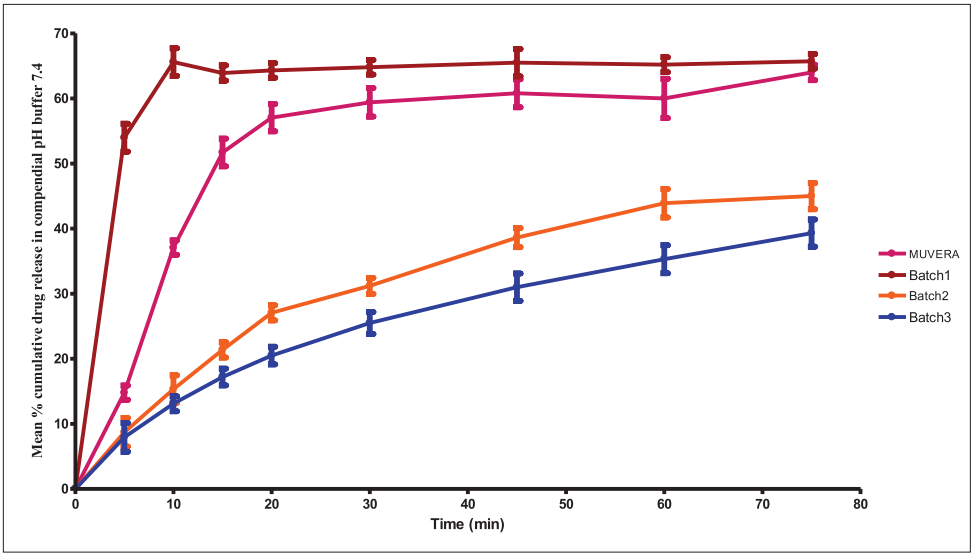


Fig. 5: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in compendial pH buffer 7.4

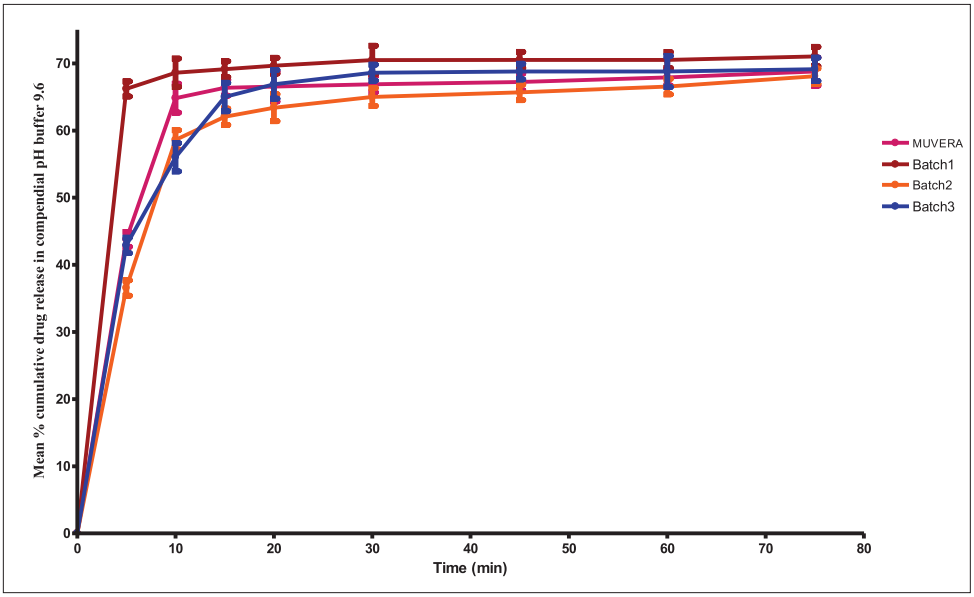


Fig. 6: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in compendial pH buffer 9.6

from Batch 2, 16.8% from Muvera® and 13.4 % from controlled Batch in 75 minutes. Almost the same results were obtained when studies were carried out in FeSSIF (pH 5.0) for all formulations (Fig. 8). In this case also, the Batch 2 has shown a poor drug release profile that may be because of the presence of DCP. However, the dissolution study in FaSSIF (pH 6.5) has shown a drastic change in drug release of Batch 1, 70.3% drug release of Batch 2, 88.0% drug release of Muvera® and 27% drug release of controlled in 75 minutes (Fig. 9).

As it is a well-known fact that bio relevant media are considered the most predictive media for *in vitro in vivo* correlation (IVIVC) studies during drug development studies that simulate gastrointestinal fluid more accurately [40]. Therefore, it can be considered that the results obtained by carrying out dissolution studies in FaSSGF (pH 1.8), FaSSIF (pH 6.5), and FaSSIF (pH 5.0) will be the surrogate marker for meloxicam nanoparticulated tablet performance inside the body. Thus, the results shown by FaSSGF (pH 1.8) revealed that meloxicam is a pH dependent poorly soluble drug and is a weak acid. Moreover, the results observed with FeSSIF (pH 5.0) showed that food is having a drastic effect on drug release of meloxicam nanoparticles as shown in Fig. 8. The drug release in FeSSIF pH 5.0 is almost 50% to that of FaSSIF (pH 6.5). Thus, it can be considered that presence of food may cause dissolution limited absorption of meloxicam nanoparticles from its tablet dosage form and thereby decrease in the therapeutic potential of meloxicam. Hence, FaSSIF (pH 6.5) could be used for IVIVC of meloxicam nanoparticles when human volunteers/animals are in fasting conditions and FeSSIF (pH 5.0) when human volunteers/animals are in fed state. "However, these media look good when normal research and development is concerned but due to their complex composition, availability of costly surfactants (sodium taurocholate and egg lecithin) and questionable storage stability, these media are expensive and their use is limited as a regular quality control medium"[35,39]. For example, 1 L of FeSSIF can cost as much as the US $ 700 [39]. This warrants to develop a single dissolution test medium which can work almost like biorelevant media during the early phase drug development process as well as for regular quality control purpose. "The replacement of natural bile components (sodium taurocholate and lecithin) with different simple testing media like milk and biphasic media may be the alternative approach for this issue" [41].

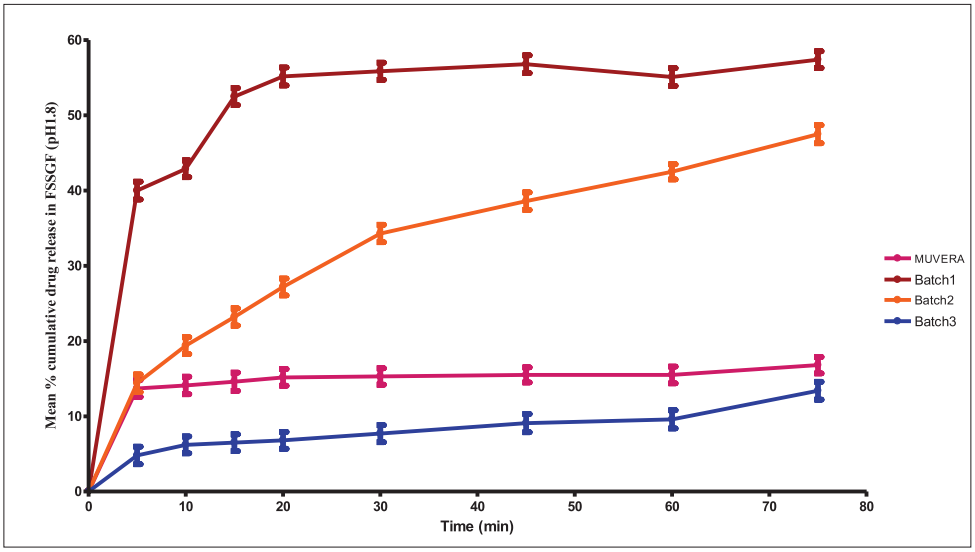


Fig. 7: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in fasted state simulated gastric fluid

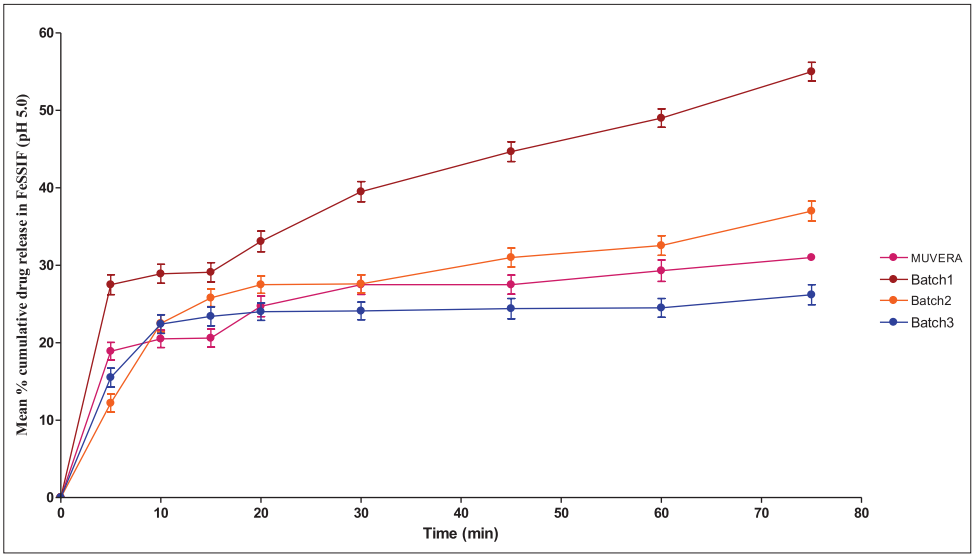


Fig. 8: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in fed state simulated intestinal fluid

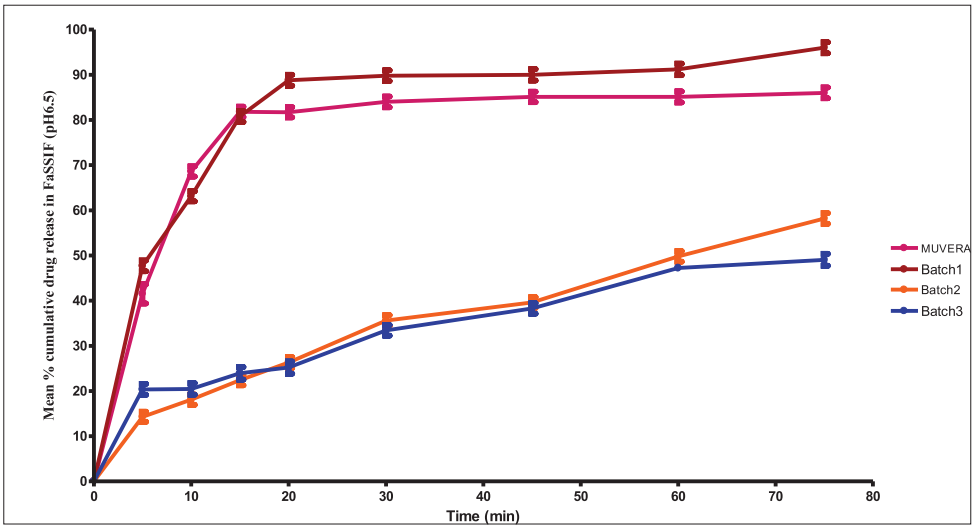


Fig. 9: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in fasted state simulated intestinal fluid

Milk as dissolution medium

An attempt has been taken by preparing SGF containing 50% milk (whole fat), SGF containing semi skimmed milk or skimmed milk. The pH of each medium was adjusted to 3 as shown in Fig. 10. For Batch 1, 45.20% drug release was observed with skimmed milk, 55.62% drug release with semi-skimmed milk and 65.36% drug release with whole fat milk, respectively, in 75 minutes, whereas 38.39% drug release for Batch 2 in skimmed milk, 40.20% drug release in semi skimmed milk, and 48.32% drug release with whole fat milk in 75 minutes, respectively.

The results showed that as the fat content was increasing in the media; the dissolution rate of meloxicam nanoparticles was increasing for both batches. Thus, it provides a clear evidence of an increase in dissolution rate of meloxicam nanoparticles tablets in milk containing media. Since, meloxicam is a lipophilic drug (BCS class II), the increased fat content of milk would be expected to aid solubility and thereby dissolution rate of the drug. However, these milk containing media also do not provide the drug release of meloxicam nanoparticulate tablets as per compendial requirements. Hence, our research has been moved toward the use of biphasic dissolution media.

Biphasic medium

The potential of the biphasic media using n-octanol has been reported by Heigoldt *et al.* [41] for predicting *in vivo* performance of Investigational New Drug BIMT 17. Hence, we have tried to explore the potential of biphasic dissolution media for providing a similar dissolution profile as that of FeSSIF (pH 5.0) and FaSSIF (pH 6.5). The choice of n-octanol as the organic phase in the biphasic system described in this report was based on its advantageous physical/chemical properties [42,43], like: (1) n-octanol is practically insoluble in water (0.05 g/100 g H_2O); (2) n-octanol is less dense than water (specific gravity 0.825 at 20°C), permitting ease of sampling; (3) n-octanol possesses low volatility (b.p.=195°C) hence, n-octanol will not readily evaporate at 37°C, and thus, a relatively constant upper phase can be maintained; (4) n-octanol possesses rather low viscosity, enabling sampling via conventional tubing and pump; (5) meloxicam used in this study, is readily soluble in n-octanol and possess octanol/water distribution coefficients with log P>3.43 (Table 1), guaranteeing sink conditions in the n-octanol layer. By this means, dissolved drug was distributed to the organic layer, removed from the aqueous dissolution medium, and a quasi-sink was obtained throughout the experiment [41].

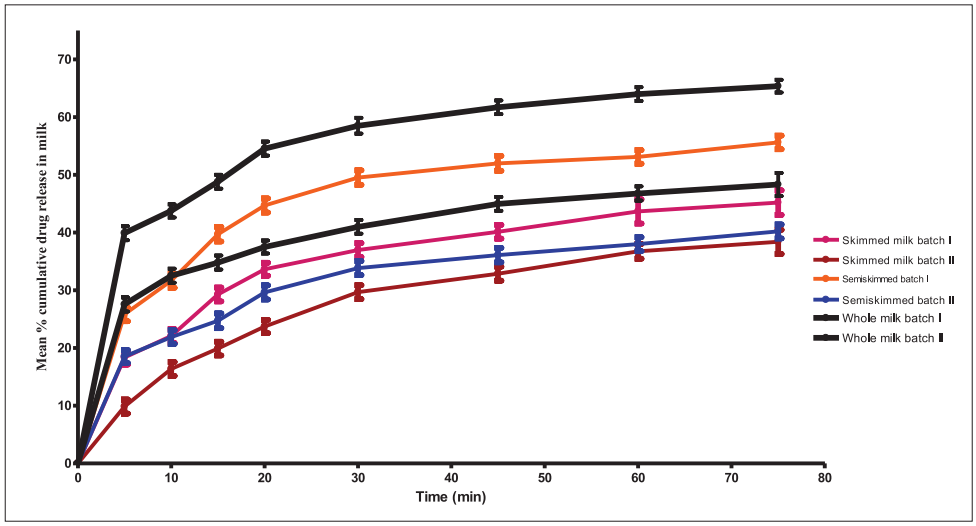


Fig. 10: Dissolution profile of Batch 1 and Batch 2 in skimmed, Semi skimmed and whole fat milk

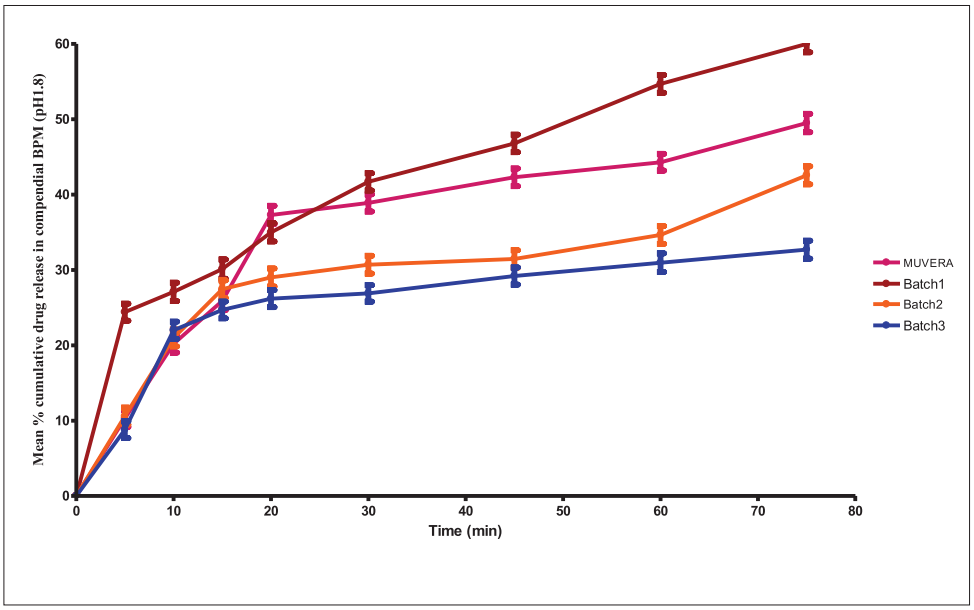


Fig. 11: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in biphasic media of pH 1.8

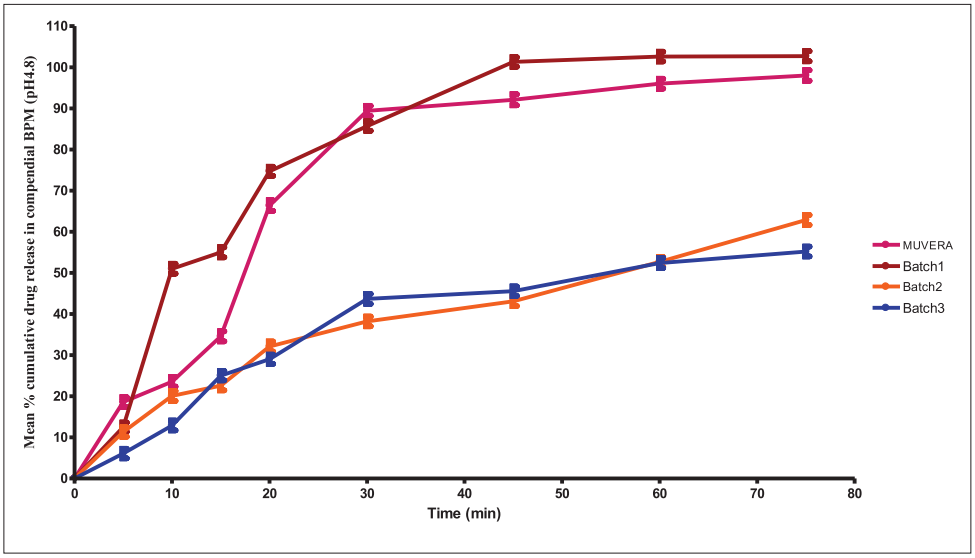


Fig. 12: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in biphasic media of pH 4.8

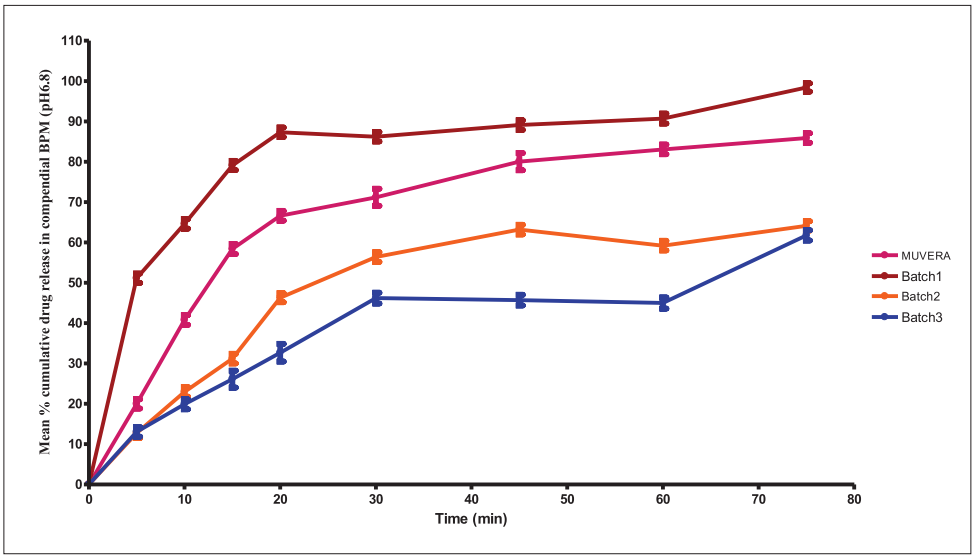


Fig. 13: Dissolution profile of Muvera®, Batch 1, Batch 2, and controlled batch (Batch 3) in biphasic media of pH 6.8

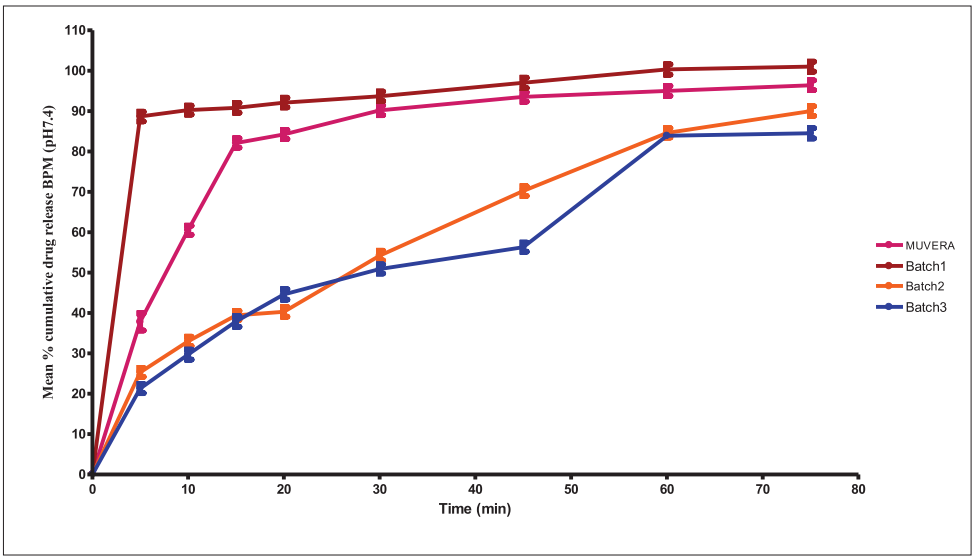


Fig. 14: Dissolution profile of Muvera®, Batch 1, Batch 2 and controlled batch (Batch 3) in biphasic media of pH 7.4

In view of this, dissolution profiles of biphasic media at different pH 1.8, 4.8, 6.8 and 7.4 (Figs. 11-14) were compared with FaSSIF and FeSSIF statistically using model independent method (Similarity factor F2). It was found that Batch 1 was giving similarity factor of 59.94 between FaSSIF and biphasic (pH 6.8) media and 51.71 between FeSSIF and biphasic 1.8 media, respectively. On the other hand, similarity factor for Batch 2 was calculated and found to be 56.28 between FeSSIF (pH 5.0) and bipahsic pH 1.8, and 51.37 between FaSSIF (pH 6.5) and biphasic (pH 4.8) media. This revealed that biphasic media of pH 1.8, 4.8 and 6.8 can be used as a surrogate marker for replacing biorelevant media. However, the medium of pH 6.8 has shown the best dissolution profile. Hence, during drug development studies, instead of using costly biorelevant media, it is better to use a simple biphasic medium which simulates *in vivo* conditions and helps in predicting *in vivo* drug performance of meloxicam nanoparticulate tablets.

CONCLUSION

In this study, the nanoparticles of meloxicam were prepared using wet media milling and the milled samples were dried using spray drier. The dried nanoparticles were converted into tablet dosage form by varying the type of diluent. The study revealed that in all the cases the nanoparticulate tablets of Batch 1 have given increased dissolution profile as compared to marketed formulation (Muvera®), Batch 2 and controlled tablets of meloxicam. This proved that the excipients also play a major role in the release behavior of drug otherwise if it was not so, the nanoparticulate tablets of Batches 1 and 2 would have given the same dissolution profile in all the tried media. Batch 1 containing lactose with higher surface area provided more and rapid wetting of the drug by the dissolution media compared to Batch 2 that contained DCP as a major diluent. Among all the dissolution media tried to evaluate the discriminatory power and simulation with biorelevant medium, biphasic medium of pH 1.8, 4.8 and 6.8 have promised to simulate with biorelevant media. However, the medium of pH 6.8 has shown the best dissolution profile. In future, *in vivo* pharmacokinetic study is required using a suitable animal to confirm the correlation of *in vitro* study with *in vivo* milieu.

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