The predictive power of biphasic dissolution approach using Class IV model drug

Mohammed Abdulzahra Hussein*, Mohanad Naji Sahib**
Department of Pharmaceutics, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq
**** Faculty of Pharmacy, Al-Turath University College, Baghdad, Iraq***

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Corresponding Author email:
mabd91382@uomustansiriyah.edu.iq
orcid: https://orcid.org/0000-0001-5790-2388

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Abstract:

This study was aimed to evaluate biphasic dissolution system and its applicability to discriminate between different formulas. Two different tablet formulas of furosemide were prepared using dry compression (F1) and wet granulation (F2). The prepared formulas were evaluated for hardness, friability and disintegration. Thereafter, monophasic and biphasic dissolution systems were used to compare the dissolution profiles of the prepared formulas with a commercially available tablet. The results of the physical properties of the prepared tablets were within acceptable values. Moreover, there were insignificant differences (P>0.05) between generic product and the prepared formulations. The similarity and difference factors were > 58 and <10, respectively. On the other hand, the biphasic dissolution system results showed significant difference regarding dissolution profiles for all items under investigation. In conclusion, biphasic dissolution system could be a viable tool in assessment in-vitro drug performance as a result of its good discriminatory power.

Key words: Two phase, dissolution, similarity factor

القدرة التنبؤية لنهج الانحلال ثنائي الطور باستخدام نموذج الدواء من الفئة الرابعة

محمد عبد الزهرة حسين*، مهند ناجي صاحب **
الجامعه المستنصريه ـ كلية الصيدلة ـ فرع الصيدلانيات (بغداد-العراق)
**** كلية التراث الجامعة ـ قسم الصيدلة (بغداد-العراق)***

الخلاصة:

كانت هذه الدراسة تهدف إلى تقييم نظام الانحلال ثنائي الطور وانطباقه للتمييز بين الصيغ المختلفة. تم تحضير تركيبتين لوحيتين مختلفتين من الفوروسيميد باستخدام الضغط الجاف (F1) والحبيبات الرطبة (F2) والطرق. تم تقييم الصيغ المحضرة من حيث الصلابة والتفتت والتفكك.بعد ذلك، تم استخدام أنظمة الانحلال الأحادي والذوبان ثنائي الطور لمقارنة ملامح الانحلال للصيغ المعدة مع جهاز لوحي متاح تجاريًا. كانت نتائج الخصائص الفيزيائية للأقراص المعدة ضمن القيم المقبولة. علاوةً على ذلك، هناك اختلافات ضئيلة بين المنتج العام والتركيبات المعدة. كانت عوامل التشابه والاختلاف > ٥٨ و < ١٠ على التوالي. من ناحية أخرى، أظهرت نتائج نظام الانحلال ثنائي الطور اختلافًا كبيرًا فيما يتعلق بملفات تعريف الحل لجميع العناصر قيد التحقيق. في الختام، يمكن أن يكون نظام الانحلال ثنائي الطور أداة قابلة للتطبيق في تقييم أداء المخدرات في المختبر نتيجة لقدرته التمييزية الجيدة

الكلمات المفتاحية: مرحلتان، الانحلال، عامل التشابه

Introduction

The Biopharmaceutics Classification System (BCS) has been used extensively in the pharmaceutical industry. Drug product development and regulation have received an advantage greatly from this categorization system, which accounts for the two most important characteristics impacting oral drug absorption: solubility and intestinal permeability [1].

Classes II and IV of the BCS will comprise acid (a), base (b), and neutral (c) sub-specifications. Sub classification for Classes I and III (drugs with high solubility as presently defined) is often unnecessary, with the possible exception of circumstances where solubility is borderline. It is well-known that the, pKa and physical property of a drug, have a significant impact on the aqueous solubility and dissolution of drug from the drug product both in-vitro and in-vivo for BCS Class II and IV [2].

In-vitro dissolution test is a crucial tool in quality control protocol. Many dissolution settings were approved to overcome some issues regarding dosage design. However, these systems were either expensive or had no discrimination power. Therefore, the need to develop simple, yet, sensitive method to be used in quality control and/or drug development was an urgent need. It has been recommended that the optimal technique for testing the drug release formulations with poor water solubility properties should be reasonably simple and affordable. In addition, dissolution conditions should be replicate as precisely as possible the physiological environment of the human gastrointestinal system [3]. Although it is challenging to meet all of these conditions in monophasic dissolution model, the biphasic dissolution test represent a good and reasonable model [4]. Levy's proposed that an upper organic phase, in addition, to aqueous phase might serve as a reservoir for dissolved drug and represent the absorptive site for the dissolved drug [5]. Till now, the biphasic dissolution system is not approved by FDA due to the paucity in the tested model drugs and standardized method.

Furosemide ($C_{12}H_{11}ClN_2O_5S$) is a sulfonamide diuretic with a powerful and short-term diuretic action. The available data on solubility, oral absorption, and permeability are sufficiently conclusive to classify furosemide into class IV according to (BCS). The aqueous solubility of furosemide at room temperature has been reported to be 0.01825 mg/ mL. Its aqueous solubility increases as function of the pH of the medium from 0.18 mg/mL at pH 2.3 to 13.36 mg/mL at pH 10 [6]. Furosemide is a weak acid with an acidic pKa value of 3.8 [7]. Moreover, the Log P (n-octanol/water) values are 2.2935 and 1.8136 have been reported [8]. All these information make furosemide a good candidate to be used in biphasic dissolution test. Therefore, the main objective of this research was to assess the applicability of biphasic dissolution test to discriminate between two manufacturing process, using furosemide as a model drug, in comparison to marketed product.

Materials and Methods

Materials

The materials used in this study as follow: A pure furosemide is a gift from Awamedica Company for Drug Industries and Medical Applications (Awa, Erbil, Iraq). Avicel PH102 (microcrystalline cellulose) is a gift from Pioneer Co. for pharmaceutical industries (Sulaymaniyah, Iraq). Other materials were purchased from its corresponding company. Crosc-armellose sodium NF from (JRS Pharma, Rosenberg, Germany). Magnesium stearate and Starch 1500 from H.L. Blachford Ltd. (Mississauga, Canada) and Colorcon (Indianapolis, USA), respectively. 1-Octanol, Ethanol 99% and ethanol 70% (v/v) (EtOH) from Sigma Aldrich (Chemie GmbH, Steinheim, Germany). Hydro-chloric acid (HCL) from (CHD (P) LTD.

INDIA). Disodium hydrogen phosphate (Na2HPO4.2H2O) and Sodium dihydrogen phosphate (NaH2PO4.2H2O) from (Thomas Baker (Chemicals) Pvt. Ltd. – India). Sodium hydroxide (NaOH) and Sodium lauryl sulfate (SLS) from (Carl Roth GmbH & Co., Karlsruhe, Germany).

Methods

Calibration curve furosemide

Furosemide calibration curves were generated in a variety of media, including phosphate buffer pH 6.8 plus sodium lauryl sulfate (SLS), and 1-Octanol and aqueous solution containing 80% (v/v) methanol with ʎmax equal to 276.8nm, 276nm and 277.1 nm, respectively. The absorbance measurements were carried out using a UV-spectrophotometer (Shimadzu, Japan) [9].

Prepare furosemide immediate release formulations

By using different manufacturing processes, two formulations were developed for this investigation. In addition, microcarystalline cellulose was selected as the neutral diluent in those two formulations to exclude any change in the microclimate environment around the drug during dissolution process. Tablets were manufactured by direct compression and wet granulation in order to investigate the manufacturing process effect. The direct compressed tablets (D) were made by combining all the materials (excluding the lubricant) in a mortar and pestle for six minutes, until a uniform consistency was achieved. Magnesium stearate was added last to prevent the coating of the active pharmaceutical ingredient (API). The wet granulation tablets (G) were made by combining all the components in the same way as D. 70% ethanol was employed as the granulation solution due to:

1-better for moisture sensitive products

2-volatile and so they evaporate/dry more quickly

, and an N60 sieve was used to granulate the wet powder combination. The granules were dried in an oven at 37 °C for one hour and sieved again using an N60 sieve. Then, the lubricant (magnesium stearate) was added and mixed for an additional minute. Each formula was listed in Table 1. In addition, a commercial marketed product was used for comparison (AwaFurosem®, Awamedica Company, Iraq).

Table (1): Furosemide formulas according to manufacturing methods.

Components	Direct compression method (D) (F1)	Wet granulation method (G) (F2)
Furosemide	40mg	40mg
MCC AvicelPH102 (Diluent, disintegrant)	96% (250mg)	84% (220mg)
Mg Stearate (lubricant)	2% (5mg)	1.5% (3mg)
CS (binder, disintegrant)	2% (5mg)	3.5% (9mg)
Starch 1500(diluent, Disintegrant, glidant)	----	11% (28mg)

MCC: microcrystalline cellulose
CS: croscarmellose sodium
$CaHPO_4$: dicalcium phosphate dihydrate
D: direct compression
G: wet granulation
Overall weight of tablet is 300mg.

The physical properties of furosemide immediate release tablets.

Hardness test

The average hardness was determined by measuring the hardness of three tablets made from different formulations. A Monsanto hardness tester (Guoming, India) was used to measure the amount of force (in kilograms per square centimeter) needed to crush the tablet [10].

Friability test

Twenty tablets were weighed, then placed in the friabiliator (Vanguard, USA), and rotated for (four minutes) at 25 revolutions per minute (rpm) to determine the tablets' friability. Tablets were de-dusted and re-weighed after the rotations. The following calculation was used to determine whether the level of friability is less than 1% [11]:

% Friability = [(weight (initial) – weight (final)) / weight (initial)] *100%

Disintegration test

A disintegration tester (Vanguard, USA) was used with Hydrochloric Acid Solution 0.1 M as a media. A selection of six pills was made at random. When there were no more pill fragments visible in the instrument, the disintegration time was considered to have been complete [12].

Determination of drug content

40 mg of tablets were dispersed in 100 ml of an aqueous solution containing 80% (v/v) methanol. The samples were sonicated for 30 minutes, and then put overnight in a shaker. The solution was filtered using a 0.22 µm membrane filter, and drug amount was measured using a UV spectrophotometer at 276 nm and (Shimadzu, Japan). Each formulation was analyzed in triplicate [13].

In vitro dissolution tests

Monophasic dissolution test under sink conditions

Dissolution investigations were conducted in 900 ml phosphate buffer pH 6.8 plus 0.5% w/v sodium lauryl sulfate (SLS) at 100 rpm and 37 °C in a USP II apparatus (Cosmo lab. Equipment , India) using the USP technique (n = 3) [14]. Aliquots of five ml were collected through 0.45 µm syringe filters at predetermined time intervals (from 1 to 60 minutes) and replaced with fresh medium. Furosemide concentrations were analyzed using UV spectrophotometer (Shimadzu , Japan) [15].

Biphasic dissolution test

The biphasic dissolution experiments were conducted in 250 ml glass beakers containing 100 ml phosphate buffer and 20 ml 1-octanol (10:2) at 37 °C as previously reported [16]. At predefined intervals (same as monophasic dissolution), the dissolution samples were withdrawn from the aqueous and oil phases using syringe filters (0.45 µm) and analyzed using UV-spectrometry spectrometer [15].

Statistical Analysis

Several statistical approaches were used in this study. When appropriate, one-way ANOVA, paired t-test and analysis of variance (ANOVA) with repeated measurements were utilized[17]. In addition, the DDSolver software was used to calculate the similarity, dissimilarity, and 90% confidence interval difference for the dissolution profiles [18]. 95% was not used because if we calculate a 95% confidence range for 100 samples, 95 of them will include the real mean value (µ). Nevertheless, we choose one random sample and construct one confidence interval that may or may not include the real mean. The interval may over- or underestimates (µ). The 95% CI represents the true, unknown parameter's probable range. Unknown parameter variability is not reflected in the confidence interval. It shows the sample's random error and gives a range of values that may include the unknown parameter. A confidence interval is the range of probable parameter values (point estimate + margin of error)

with a set confidence level (which is similar to a probability)[19].

Results and Discussion

Calibration curve of furosemide

The furosemide concentrations in phosphate buffer solution and 1-octanol produced straight lines with a high correlation coefficient (0.9994 and 0.9989, respectively).

Physical properties of tablet formulations

Furosemide immediate release tablets

All prepared formulas have good hardness (less than 7 kg/cm^2), low friability (<1%) which resist the mechanical shocks [20]. Moreover, the results showed acceptable disintegration time results (less than 8 minutes) [21]. The results showed insignificant difference in hardness and friability tests for both formulations compared to AwaFurosem®. Furthermore, the results of the disintegration time showed insignificant (F1) and significant (F2) differences compared to marketed product. However, these results were within acceptable values (Table 2). Moreover, the results showed insignificant differences in drug content for all formulas and marketed drug (P>0.05).

Table (2): Physical properties of furosemide immediate release formulations (mean± standard deviation).

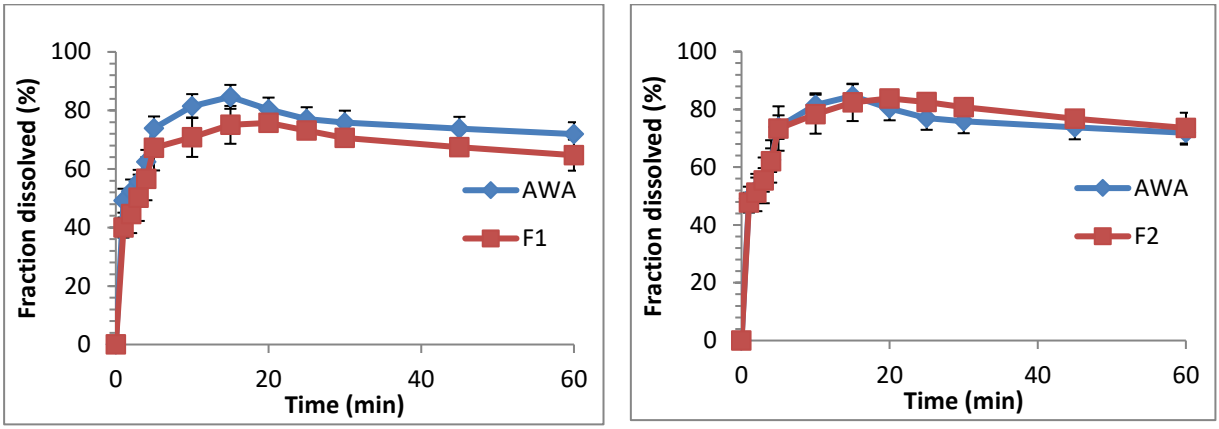
Formulas	Hardness (kg/cm^2)	Friability (%)	Disintegration Time(min)
F1	5.75±0.49	0.76±0.01	7.53±0.03
F2	6.60±0.28	0.64±0.03	6.49±0.14**
AwaFurosem®	6.15±0.63	0.74±0.04	7.54±0.37

** *P<0.01*; Formulas:
F1: direct compression formula.
F2: wet granulation formula,
AwaFurosem® (control; marketed product**).**

In-vitro dissolution tests

Monophasic dissolution test for furosemide

The dissolution profiles of furosemide, (AwaFurosem®) and prepared the formulas, in phosphate buffer pH 6.8 plus SLS were shown in Figure 1. More than 70% of furosemide was released within 20 minutes. The results showed insignificant differences (P>0.05) in release time ($t_{25\%}$, $t_{50\%}$, and $t_{75\%}$) between finished product and the prepared formulas of furosemide as shown in Table 3.



AWA: finished marketed product
F1: direct compression
F2: wet granulation

Figure (1): In-vitro furosemide release profiles (monophasic dissolution) of AwaFurosem® (AWA) and formulas (F1 and F2) in compendial buffer (50mM phosphate buffer pH 6.8 plus SLS in 900ml).

Table (3): Release time of finished product and formulas of furosemide (Mean percentage of labeled amount dissolved ± SD).

Parameter	Release time of formulas of furosemide		
	F1	F2	AwaFurosem®
$T_{25\%}$	0.985±0.98	0.943±0.95	0.957±0.97
$T_{50\%}$	1.470±1.03	1.336±0.96	1.213±0.98
$T_{75\%}$	33.108±1.26	30.351±1.4	34.212±0.96

F1: direct compression
F2: wet granulation

Moreover, the difference factor values between AwaFurosem® and formulas were less than (15) and the similarity factor values were more than (50) as shown in Table 4. Although different manufacturing processes were used, all profiles were similar in the conventional USP II dissolution test.

Table (4): Compare the dissolution profile between furosemide formulas and AwaFurosem® using mathematical methods ($\int_1$ and $\int_2$)

Comparison	$\int_1$ ± SE	$\int_2$ ± SE
AwaFurosem® × F1	9.80 ± 3.65	58.38 ± 2.41
AwaFurosem® × F2	3.31 ± 0.51	76.37 ± 1.12

$\int_1$: difference factor
$\int_2$: similarity factor
SE: standard error

The release model was Weibull model for all formulations and the finished product (Table 5) depending on the previous reported criteria [18, 22]. The overall results revealed that the monophasic dissolution method showed a low discriminatory power

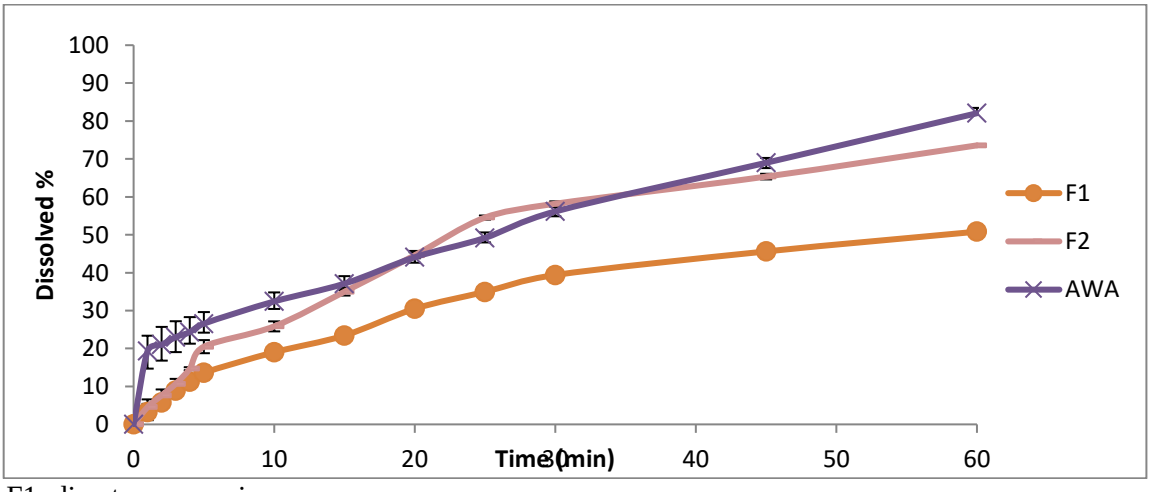
Table (5): Model dependent approach of furosemide formulations and AwaFurosem®.

Model	Statistics	Formulas		
		F1	F2	AwaFurosem®
Zero order	R^2-adj	-3.26	-2.86	-2.91
	AIC	134.13	132.23	132.76
	MSC	-2.75	-2.50	-2.53
First order	R^2-adj	0.33	0.18	0.26
	AIC	109.91	112.00	110.99
	MSC	-0.89	-0.94	-0.85
Higuchi	R^2-adj	-0.71	-0.49	-0.52
	AIC	122.31	119.93	120.51
	MSC	-1.84	-1.55	-1.59
Weibull	R^2-adj	0.89	0.89	0.89
	AIC	87.74	87.30	87.21
	MSC	0.81	0.95	0.96
Korsmeyer-Peppas	R^2-adj	0.87	0.85	0.86
	AIC	89.30	90.19	90.16
	MSC	0.69	0.73	0.74

Bold font represents the best model
F1; direct compression
F2; wet granulation
R^2; Adjusted correlation coefficient
AIC; Akaike Information Criterion
MSC; Model Selection Criterion

Biphasic dissolution for furosemide formulas

Figure 2 shows the overall release of the finished product (AwaFurosem®) and prepared formulas (F1 and F2). The overall release of the AwaFurosem® was more than 80%, while, the release of the prepared formulas was ranked as follow: F2 (>70%) > F1 (>50%). The overall release percent were ranked as follow: commercial product > wet granulation tablet formula > direct compression tablet formula.



F1: direct compression
F2: wet granulation

Figure (2): Overall release profile for finished and prepared formulas of furosemide.AWA: finished marketed product (AwaFurosem®)

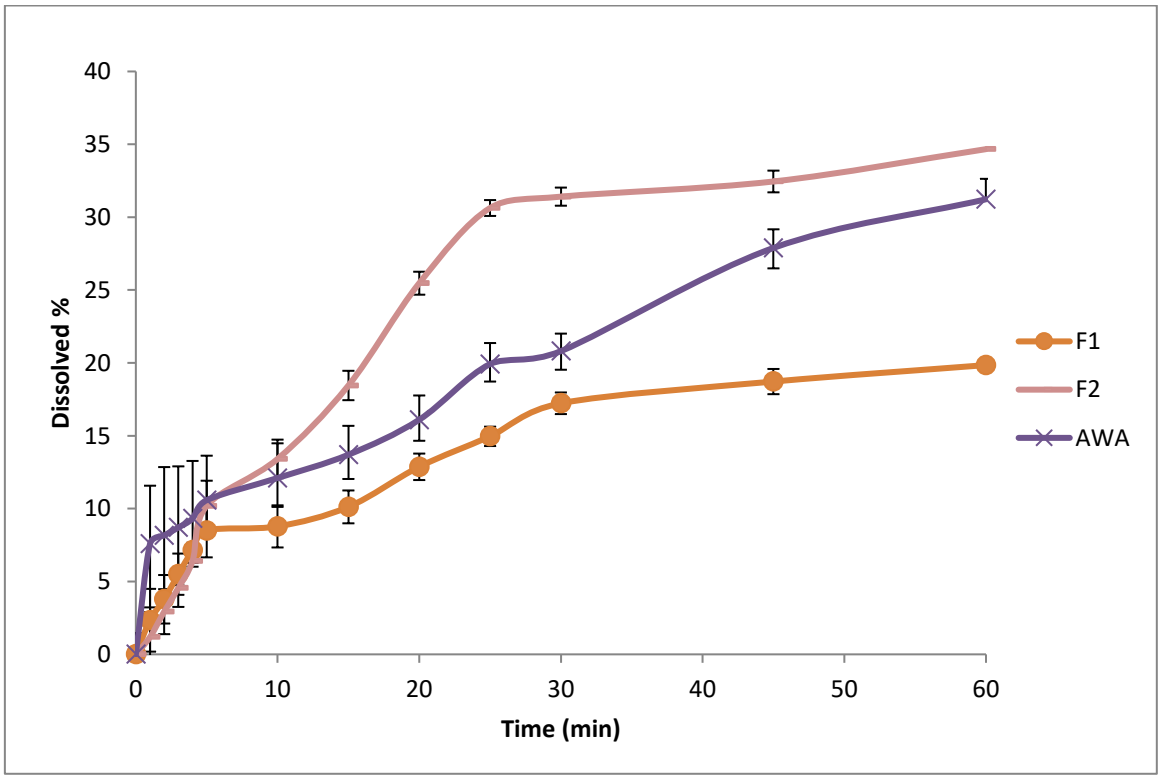
In aqueous media, the results revealed that F1 failed to be similar to the control formulation (AwaFurosem®), however, F2 was similar. In a similar manner, the findings of the organic phase showed rejection in similarity for all formulations, as shown in Table 6.

Table (6): 90% confidence interval difference of biphasic dissolution profile for different formulas of furosemide. Biphasic media ratio 10:2 (100 ml phosphate buffer (50mM pH 6.8): 20ml octanol).

Formulas	Reference product (AwaFurosem®)	
	Aqueous phase	Organic phase
F1	R	R
F2	A	R

R; Reject or A; Accept.
F1: direct compression
F2: wet granulation

Using repeated measure ANOVA (We used similarity and difference factors in comparison for monophasic dissolution only). showed highly significant differences between prepared formulas (F1 and F2) and commercial product. To assess the effect of manufacture process on the release profile, only the release in the aqueous phase was assessed. The effect of manufacture process was pronounced and the results reveled that wet granulation method showed higher release profile (P<0.001) as shown in Figure 3.



AWA: finished marketed product (AwaFurosem®)
F1: direct compression
F2: wet granulation

Figure (3): Furosemide release profile in biphasic aqueous media for formulas (F1 and F2) with different manufacturing process compared to AwaFurosem®.

Moreover, the release profile of the direct compressed formula was significantly lower than other formulas. These results may be due granulation process and the final wettability of the granules [23, 24]. These results indicated that the manufacturing process which is an important variable in drug product development can be easily discriminated by biphasic dissolution system [25] [16].

The results revealed that the biphasic dissolution was superior over monophasic dissolution in discrimination power between different formulas and could be used in quality control and drug development process. We cannot combine two figures because in monophasic dissolution the media was aqueous only but in biphasic the media was mixed (aqueous and organic) so, measured aqueous only.

Conclusion

Manufacturing process and subsequent quality control need approaches with high specificity and selectivity to understand the overall difference between manufacturing variables. This project revealed the usefulness of using biphasic dissolution system. The results showed excellent discrimination power between direct compression and wet granulation processes in comparison to marketed product. However, there is a need for more effort to standardize its applicability to cover more variables and/or other model drugs.

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