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Development of metformin tablet that mimics the circadian variation of glucose levels

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Halle, April 23rd, 2013

Dedication

To Professor Dittgen, for his support and guidance without which this work couldn't be possible.

To my wife Serin, for her love, care, and patience.

To my parents, sisters and brother for standing there for me.

Contents

Abbreviations

CA	Citric acid
CMTG	Carboxyl methyl ether of TSG
EFT	Effervescent floating tablet
FLT	Floating lag time of tablet
H	Tablet hardness
HCl	Hydrochloric acid
HPMC	Hydroxypropyl methylcellulose
HPTG	2-hydroxypropyl ether of TSG
M	Metformin hydrochloride
Ph. Eur.	Pharmacopoea Europaea
PVP	Poly(vinyl pyrrolidone)
rpm	Rotations per minute
S	Percent of tablet thickness to it is initials after swelling for $t_{60\%}$.
SA	Stearic acid
SB	Sodium bicarbonate
$t_{60\%}$	Time needed to release 60% of drug content form tablet
TSG	Tamarind seed gum
USP	United States Pharmacopeia
W_{gm}	Work of adhesion of table on rabbit gastric mucosa
W_{ss}	Work of adhesion of tablet on stainless steel

1 Introduction

Metformin is a hypoglycemic agent widely used in clinical practice for more than half decade to treat diabetes. It is safe and effective as mono-therapy or can also be used in combination with any other hypoglycemic agent for treatment of diabetes (Ali and Fonseca 2012). The preferential action of metformin in hepatocytes is due to the predominant expression of the organic cation transporter 1 (OCT1), which has been shown to facilitate cellular uptake of metformin (Shu et al. 2007). In liver and other organs metformin induces mild and specific inhibition of the mitochondrial respiratory chain complex 1 (El-Mir et al. 2000). As result, decrease glucose production in the liver and glucose absorption in the intestine; increases the affinity of the insulin receptor for insulin. Consequently, it increases the uptake and utilization of glucose by skeletal muscle and adipose tissues. Finally, the insulin secretion is decreased. The lowering of blood glucose levels by metformin is only observed in people with diabetes and insulin resistance with an advantage of no weight gain and lower risk of hypoglycemia in patients.

In 2006, pharmacy retailers began offering low cost generic medications at costs ranging from $4 for a 30-day supply to $10 for a 90-day supply because of expire of the original patents (Butler et al. 2012) However, recent report shows that the global type 2 diabetes therapeutics market was worth $23.7 billion in 2011. By 2020, the market is expected to be worth $45.1 billion (Bombourg, 2012). Metformin, alone or in combination with other oral agents, has become the standard of care for the first-line treatment of type 2 diabetes patients.

There are numerous solid dosage forms containing metformin currently available in the market (Table 1.1). Besides the immediate releasing (IR) tablets four types of extended release (XR) tablets are sold.

Table 1.1 Currently marketed solid dosage forms containing metformin (Physicians' Desk Reference online)

Brand name (Doses)	Drug release, technological principle (Brand)	Owner of technology
Glucophage® tablets (500mg, 850mg,1000mg)	IR, film coated tablet	Merck
Fortamet® tablets (500mg,1000mg)	XR, osmotic principle (SCOT®)	Andrx
Glucophage® XR Tablets (500mg, 750mg)	XR, matrix tablet (Gel Shield Diffusion System®)	Merck-Lipha*
Glumetza® Tablets (500mg)	XR, matrix tablet (AcuForm®)	Depomed
Glumetza® Tablets (1000mg)	XR, coated tablet (Smartcoat®)	Biovail

* Bristol-Myers Squibb is licensed to distribute Glucophage® XR in U.S.A.

The osmotic principle (Fortamet®) is based on a tablet core coated with an inner sealed layer and an outer semi-permeable membrane that can take water by osmotic pressure. Accumulated pressure pushes metformin to be released from the core, at constant rate, through two laser- drilled ports (Verma et al. 2002).

The matrix tablets of Gel Shield Diffusion System® principle (Glucophage® XR) contain solid particles of retardant polymer with metformin, embedded in a continuous matrix of a second hydrophilic

polymer that enlarges in gastric fluid and prevents tablet passage through the pylorus. The tablets are gastro-retentive and metformin is gradually released by diffusion (Timmins et al. 2005).

Another gastro-retentive tablet (Glumetza® 500mg from Depomed) contains polymeric excipients that enlarge in gastric fluid and prevent tablet passage through the pylorus whilst metformin is gradually released by diffusion (Schwartz et al. 2006).

For another gastro-retentive tablet (Glumetza® 1000mg from Biovail) an outer coating layer that enlarges in gastric fluid is preventing the tablet passage through the pylorus whilst inner layer cause metformin to release gradually by diffusion (Adis R&D Profile, 2005).

After oral administration metformin is mostly absorbed in upper small intestine involving an active uptake process (Zhou et al. 2007) hence metformin in immediate release (Glucophage®) tablet will be released without been mostly absorbed which could explain its low bioavailability (60%) and its frequent gastrointestinal side effects, also, extended release metformin tablet (Fortamet®) could be administered and pass through this narrow absorption window without releasing the right amount of metformin. Gastroretentive tablets (Glucophage® XR or Glumetza®) are able to stay in stomach and release metformin during 8-9 hours (Schwartz et al. 2006).

Compliance with the metformin IR formulation can be poor, due to multiple daily dosing and frequent gastrointestinal side effects. Meanwhile XR dosage forms especially the gastroretentive tablets do have lesser side effects (Table 1.2) and administered once or twice daily.

Table 1.2. Incidence of nausea and diarrhea: comparison between metformin immediate (IR) and extended release (XR).

	IR	XR
Initial dose 1000 mg once daily in first 2-3 weeks[*]		
Nausea[†]	8.2%	3.0%
Discontinuation in first 3 weeks of treatment 1500 mg IR(am/pm) vs. XR (am/pm)[*]		
Due to Diarrhea	1.2%	0.0%
Due to Nausea	2.3%	0.0%
Discontinuation in first 3 weeks of treatment 1500 mg IR(am/pm) vs. XR once daily[*]		
Due to Diarrhea	1.2%	1.1%
Due to Nausea	2.3%	0.0%
On IR and at 6 months after switchover to once daily XR [**]		
Diarrhea[‡]	58.0%	14.0%
Nausea[‡]	18.0%	6.0%

[*] Schwartz et al. 2006 (Glumetza® vs. Metformin IR).

[†] Significant difference (α=0.05).

[**] Levy et al. 2010 (Diabex® XR or Glucophage® XR vs. Metformin IR).

[‡] Significant difference (α=0.02).

(am/pm) 500 mg in the morning and 1000 mg in the evening

The circadian timing system allows the organism to adapt its internal metabolism to changes in the external environment created by daily fluctuations in the light/dark cycle. Thus, daily circadian oscillations in many physiological parameters such as cardiovascular function,

thermoregulation, and glucose metabolism have long been characterized in mammalian physiology (Rutter et al. 2002). Misalignment of internal circadian oscillators with the external environment leads to deleterious health consequences and has long been associated with increased morbidity and mortality in humans (Reddy and O'Neill 2010). In addition, the fluctuation of blood glucose is a significant independent risk factor of mortality especial in critically ill patients (Tang and Gu 2012).

An early investigation (Rigas et al. 1968) has shown that a usual metformin treatment with 0.5 to 1.0 g metformin two or three times daily was associated with an increase in rhythmic fluctuation of blood glucose levels.

In vitro and in animals (using rat cells as model), Um et al. (2007) have proposed a molecular mechanism by which metformin causes a dramatic shift in the circadian phase of peripheral tissues. Also Caton et al. (2010) showed that metformin markedly enhanced expression of the core clock components and was associated with reduction of hyperglycemia and hyperinsulinemia in db/db mice (leptin resistant).

Taken together, the apparent beneficial association between targeted modulation of the circadian system and whole-body metabolic state suggests that chronotherapy could be a promising approach for the treatment of obesity and type 2 diabetes (Viollet et al. 2012).

Furthermore, in 2011, National Institutes of Health (NIH) associate director for science policy, Amy Patterson, M.D., mentioned metformin as a prime example of investigating new uses for already approved drug. Two-thirds of the National Cancer Institute (NCI) funded clinical trials of metformin now under way were initiated in 2011 or later (Harding, 2012). Table 1.3 shows metformin treatment benefits reported recently that could be new targets.

Table 1.3. Metformin treatment benefits reported recently.

Medical conditions	Associated conditions	References
Dyslipidemia	Type 2 diabetes	Pfützner et al. 2011
Hypercholesterolemia	Type 2 diabetes	Rosenstock et al. 2010
Prediabetes	No diabetes	Bray et al. 2012
Prevention of weight gain	No diabetes	Bray et al. 2012
Polycystic ovary syndrome	No diabetes	Tang et al. 2012
Polycystic ovary syndrome	Gestational complications	De Leo al. 2011
Gestational diabetes	Gestational complications	Gandhi et al. 2012
Cardiovascular risk	Polycystic ovary syndrome	Ziaee et. al. 2012
Heart failure	Insulin resistance	Wong et al. 2012
Body weight, blood pressure, insulin resistance, adiponectin and tumor necrosis factor (TNF)-alpha	Obese adolescents with liver steatosis	Akcam et al. 2011
Brain cancer (tumor tissue cultures for 8 patients)	Temozolomide-based chemotherapy	Soritau et al. 2011
Breast cancer	Postmenopausal women	Bershtein et al. 2012
Pancreatic cancer	Diabetes	Sadeghi et al. 2012

During recent annual meeting of the American Association for Cancer Research, Japanese scientists reported anti-proliferative action of metformin on various types of human lung cancer cell lines (Ashinuma H et al. 2012). Given the epidemiological evidence between type 2 diabetes mellitus and increased risk of cancer, the anti cancer effects could be related to the fact that several days after administering the metformin, insulin levels are reduced by 25-33% in both diabetic and non diabetic patients (Del Barco, 2011) or it could due to insulin-independent unknown mechanism (Monteagudo et al. 2012).

As mentioned above, an optimal therapy of diabetic disease using metformin alone or in combination with other oral anti diabetic should consider the circadian rhythm, and the known controlled-release or extended-release formulations as well the gastro-retentive dosage forms created so far cannot guarantee that. Furthermore, the late maximum plasma drug concentration after administration of the extended-release formulations is a disadvantage. However, twice-daily dosing with metformin IR could solve circadian problem but this approach implies considerable discomfort and the risk that patients omit the second intake. The hypothesis of this investigation is to control the fluctuation of blood glucose concentration by an optimized dosage form (Figure 1.1) that might improve the patient's outcome.

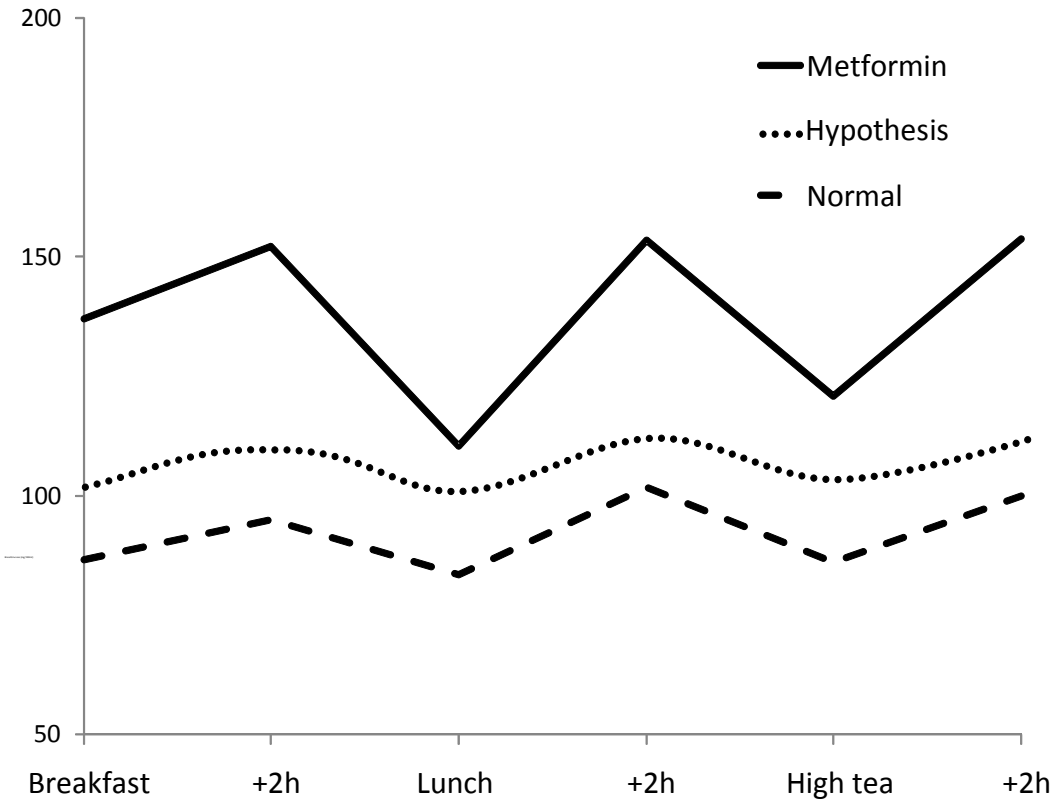

Figure 1.1. Mean circadian variation of blood glucose in normal subjects (broken line) and diabetic patient on diet and usual metformin treatment (bold line), adapted from Rigas et al. (1968), in comparison to a metformin treatment with the optimized dosage form (hypothesis, dotted line).

2 Objectives

The general objective of this work was to develop a tablet of metformin that realizes drug release according to the most modern biologic findings, which means drug release that mimics the circadian variation of glucose levels.

To realize that objective we have chosen the known effervescent floating principle as described by several authors (Bomma et al. 2009; Basak et al. 2007).

Floating tablet remains buoyant in the stomach for a prolonged period of time without affecting the gastric emptying rate of the other contents (Belgamwar and Surana 2009) which is a real risk for swelling or expandable tablet (Klausner et al. 2003). In addition, floating tablet does not have the necessity to be in contact with gastric mucosa which is a primary condition for gastric mucoadhesion tablet (Andrews et al. 2009). High density tablet or multi unit system (Jelvehgari et al. 2010; Ige and Gattani 2012; Pandit et al. 2012) will not be suitable for single high dose drug like metformin because usually they are prepared by using high ratio of excipients: drug (Timmins et al. 2005). Dosage forms that apply coating or other process that are time or money consuming were excluded because they will lead to a relative high cost for metformin treatment.

The effervescent principle is suitable for drug with high doses and high solubility like metformin because it uses a relatively small amount of gas generating agent.

It was the primary objective of our studies to vary and adapt the effervescent floating principle with regard to at least two dependent variables, drug dissolution and buoyancy. This work was done as an

optimization of a metformin effervescent floating tablet containing hydroxypropyl methylcellulose and stearic acid (Rajab et al. 2010).

The second objective was the optimization of the hot melt process to evaluate stearic acid as excipient able to control the release of metformin.

We decided to advance the effervescent floating principle by mucoadhesive properties as most recent shown by a review comprising several drugs (Prinderre et al. 2011). With respect to these properties a measuring method for adhesion on mucosa was established (Rajab et al. 2012a) and tamarind seed and poly(vinyl pyrrolidone) were considered as excipients (Rajab et al 2012b-submitted). In this context two further objectives came up, to investigate the combinatorial effects of several water soluble polymers on the in vitro performance of the floating mucoadhesive tablets and to clarify the relationship between adhesion measurement on stainless steel and mucoadhesion measurement on rabbit gastric mucosa.

A biostudy on human volunteers should show if the tablet finally chosen was bioequivalent to one of the market leaders, Glucophage® XR 750mg tablets.

3 Adaption of the effervescent floating principle

3.1 Introduction

A drug dosage form that floats immediately upon contact with gastric fluids may increase the bioavailability of drugs with absorption windows in the upper small intestine (Streubel et al. 2006). Metformin (M) absorption after oral administration is likely site dependent, with an absorption window predominantly present in the small intestine (Balan et al. 2001). Effervescent floating tablets (EFT) based on hydrophilic polymers such as hydroxypropyl methylcellulose (HPMC), further components as lipophilic matrix formers like stearic acid (SA) or beeswax, and an effervescent compound, e.g. sodium bicarbonate (SB), have been shown to have floatable characteristics (Arora et al. 2005; Basak et al. 2007). Developing such an EFT, one has to optimize the composition with regard to at least two dependent variables, drug dissolution and buoyancy. Numerous articles dealt with an optimization of EFT resulting in compositions containing 10 to 79% HPMC, 9 to 26% SA, and a time to dissolve 60% of the embedded drug ($t_{60\%}$) between 2 and 10 hours (Table 3.1).

A 32 full factorial experimental design has been used for optimization predominantly. To examine in vitro buoyancy, the method described by Rosa et al. (1994) has been commonly used (Basak et al. 2007; Prajapati et al. 2008) to determine the floating lag time (FLT). The objective of the present investigation was to optimize the composition of EFT showing acceptable hardness (H> 10 kg/cm^2) containing 500 mg M per tablet whereas the total mass should not exceed 1000 mg. In addition, the initial burst dissolution of M as a drug showing high water solubility (>300 mg/ml at 25° C) should be avoided ($t_{60\%}$ > 4.5 hours). To avoid sinking of the tablet in the stomach FLT should be shorter than 120 s. Since the pH of the stomach under fed conditions is elevated (3.5 to 4.0), citric acid (CA) should be used to provide an acidic environment for SB to ensure

Table 3.1. Review on EFT based on HPMC, drugs embedded, content of drugs, HPMC, SA and CA, experimental design so far as used to optimize the EFT, and some of the resulting dependent variables: FLT, and the time to dissolve 60% of the drug ($t_{60\%}$)

Drug	Content (mg/ tablet)	Exp. design	HPMC (%)	SA (%)	CA (%)	FLT (s)	$t_{60\%}$ (h)	Ref.
Atenolol	50	-	53–79	0	0.8–4.3	90–900	4–9	Srivastava et al. 2005
Carbamazepine	200	simplex	27–34	15*	-	153–255	7–10	Patel DM et al. 2007
Domperidone	30	BB**	10–30	-	-	2–33	>9.5	Prajapati et al. 2008
Dipyridamol	150	32	20–40	-	-	<180	6.5–10	Patel VF 2007a; 2007b
Diltiazem	240	32	25–35	15–25†	10–19#	240–1800	7.5–13	Gambhire et al. 2007
Metformin	500	-	18–22	-	1.8–2.4	15–42	2.5–3.5	Basak et al. 2007
Metformin	500	simplex	13–29	10–27	2	0–120	2–6.8	Here
Ranitidine	336	32	17	0–2	1.8–3.7	65–695	>5	Dave et al. 2004

* beeswax

** Box-Behnken

† Compritol 888 ATO

succinic acid

CO_2 generation. A simplex lattice experimental design was used as an optimization technique (Table 3.2).

Table 3.2. Simplex lattice experimental design

Formulation variables		Levels (mg)		Response variables (Y)	Constraints	
		Low	High		Min.	Max.
X_1	SA	100	270	H (Kg/cm^2)	10.2	20.3
X_2	HPMC	130	300	FLT (sec)	0	120
X_3	SB	75	245	$t_{60\%}$ (hours)	3	4.5

Using a hydrophilic compound (HPMC) and a lipophilic compound (SA) together in one matrix requires two models to decide which composition will result in Fickian diffusion or in overlapping of two dissolution mechanisms, diffusion and matrix erosion. The relative contribution of each of these two mechanisms, diffusion or matrix erosion, was quantified by applying the two terms dissolution equation, Eq. (3.1), as proposed by Catellani et al. (1988), and applied by Efentakis et al. (2007) in drug dissolution studies from hydrophilic matrices loaded with drugs of different solubility. For a given time (t), the first term of the right part of Eq. (3.1) represents the Fickian diffusion and the second term refers to matrix erosion. If this term becomes negative, there is obviously no contribution of that mechanism to the drug dissolution, and Eq. (3.1) could be reduced to Eq. (3.2) which is equivalent to Higuchi (1963) model.

$$\frac{M_t}{M_\infty} = k_1 \times \sqrt{t} + k_2 \times t \qquad (3.1)$$

$$\frac{M_t}{M_\infty} = k_1 \times \sqrt{t} \qquad (3.2)$$

Starting this investigation, we did know the design of a floatable gastroretentive tablet of M and its in vitro testing (Basak et al. 2007). Two reasons precluded us from reproducing this approach:

- The manufacturing method, a conventional non aqueous wet granulation using poly(vinyl pyrrolidone, PVP K90) in isopropyl alcohol,
- The excipient lactose as defined in the experimental part. The disadvantage of using non aqueous wet granulation is, that it has to be processed with organic solvents in particular during the granulation process, the organic solvent having to be removed again as completely as possible before granulates are processed further. For the excipient lactose, differential thermal analysis and differential scanning calorimetry thermal data showed incompatibility with M (Santos et al. 2008).

3.2 Results and discussions

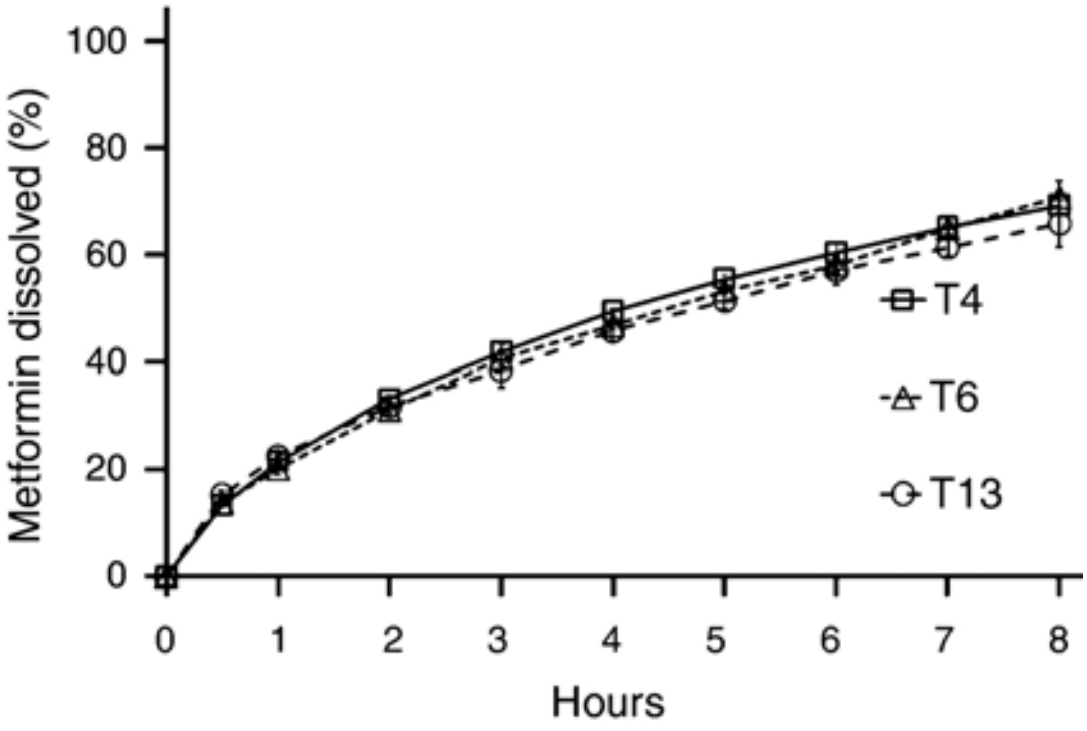

Figure 3.1. Percent of cumulative dissolution of M from EFT T4, T6, T13 (fitting data see Table 3.4).

Table 3.3. EFT compositions and experimental results of hardness (H), floating lag time (FLT) and the time to dissolve 60% of the drug ($t_{60\%}$)

No.	X_1	X_2	X_3	H±SD (kg/cm^2)	FLT±SD (s)	$t_{60\%}$ ±SD (h)
T1	270	130	75	13±1.7	135.5±11.3	1.9±0.03
T2	100	300	75	6.9±0.7	347.4±88.1	3.5±0.04
T3	100	130	245	5.4±0.7	66.7±6.8	2.5±0.07
T4	213.4	186.6	75	10.3±0.6	175.8±22.3	6.1±0.92
T5	213.4	130	131.6	11.7±0.9	209.8±59.9	3.4±0.18
T6	100	243.4	131.6	6±1.1	41±11.5	6.2±0.26
T7	156.6	243.4	75	13±0.6	304.3±44.5	3.1±0.03
T8	156.6	130	188.4	11.2±0.7	165.3±19.1	2.6±0.20
T9	100	186.6	188.4	5.6±0.9	37.2±6.2	2.0±0.04
T10	156.6	186.6	131.6	13.9±1.2	99.6±18.9	3.5±0.07
T11	128.2	243.2	103.2	7.2±1.2	39±3.5	3.2±0.08
T12	128.2	158.2	188.2	6.7±0.6	131.7±7.1	4.4±0.20
T13	213.2	158.2	103.2	13±0.3	213±42.4	6.8±0

SD standard deviation (n = 6)

EFT in all the batches prepared (Table 3.3) contained M within 100±5% of the labeled content (500 mg), and complied with pharmacopoeia specifications for weight variation (less than ±1%) and friability (less than 0.3%). The hardness (H) of the EFT was between 5.4 and 13 kg/cm^2, and FLT was found between 37.2 and 347 s (Table 3.3). Three of EFT's (T4, T6, T13) have shown an overlapping of two dissolution mechanisms, drug diffusion and matrix erosion (R^2 > 0.997, Table 3.4). The dissolution profiles of T4, T6 and T13 are quite similar (Figure 3.1).

Most of EFT released the drug following a simple Fickian diffusion mechanism (R^2 > 0.924, Table 3.5), and the dissolution profiles of these EFT's are quite different (Figure 3.2).

Table 3.4. Results from fitting the dissolution data following Eq. (3.1)

Tablet formulation	Release constant k_1	Release constant k_2	R^2	Diffusion at $t_{60\%}$ (%)	Erosion at $t_{60\%}$ (%)
T4	0.217	0.011	0.997	89	11
T6	0.189	0.021	0.999	78	22
T13	0.218	0.005	1	94	6

Checking the experimental response for hardness (H), floating lag time (FLT), and $t_{60\%}$ by a linear, Eq. (3.3) quadratic, Eq. (3.4) special cubic, Eq. (3.5), and cubic model, Eq. (3.6) using multivariate regression analysis resulted in best fit for the cubic model (Table 3.6).

$$Y= b_1X_1 +b_2X_2 + b_3X_3 \quad (3.3)$$

$$Y= b_1X_1 +b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (3.4)$$

$$Y= b_1X_1 +b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 \quad (3.5)$$

$$Y= b_1X_1 +b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + c_{12}X_1X_2(X_1 - X_2)+ c_{13}X_1X_3(X_1 - X_3)+ c_{23}X_2X_3(X_2 - X_3) \quad (3.6)$$

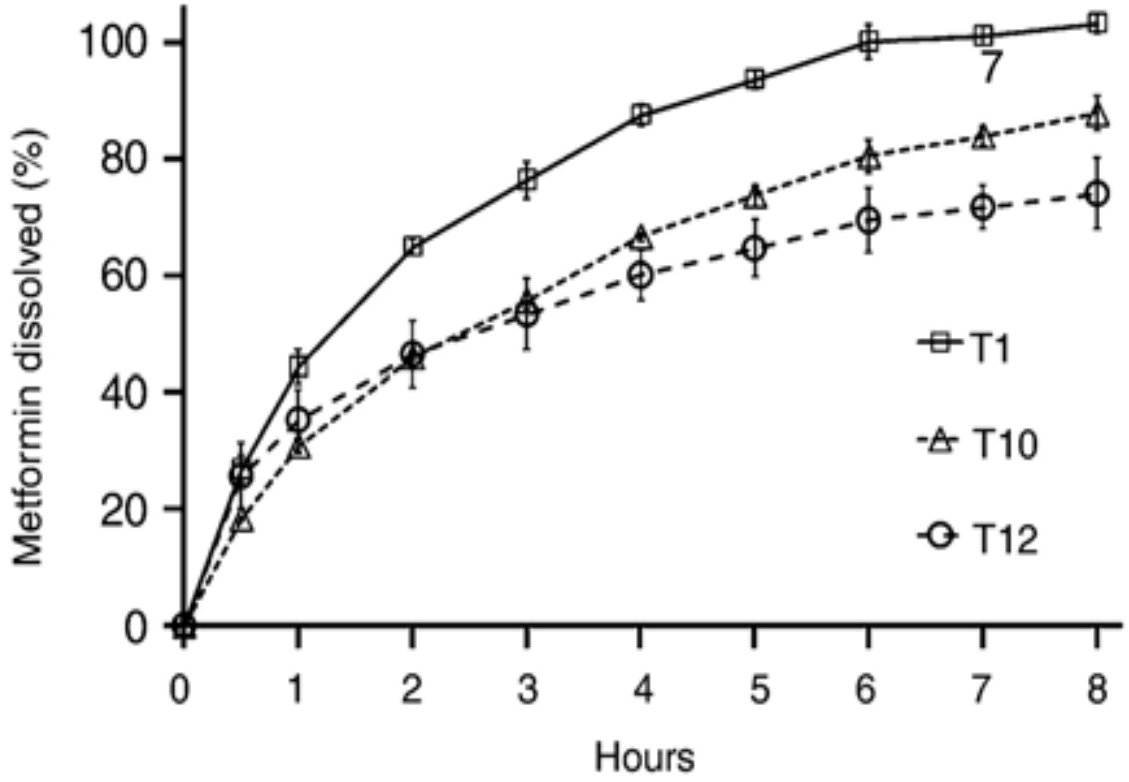

Figure 3.2. Percent of cumulative dissolution of M from EFT T1, T10 and T12 (fitting data see Table 3.5).

It was concluded from previous investigations of EFT containing HPMC (Table 3.1), to test a wide range of HPMC content between 130 and 300 mg per tablet (26 to 60% related to drug) to precise the optimum percentage of that consistency performing excipient. The EFT developed contain the highest amount of drug, 500 mg (Table 3.1), compared with previous studies and as before also achieved for M (Basak et al. 2007) but non aqueous wet granulation and the excipient lactose have been avoided.

The simplex lattice experimental design based on constraints of H between 10.2 and 20.3 kg/cm^2, FLT between 0 and 120 s and drug dissolution ($t_{60\%}$) between 3 and 4.5 h (Table 3.2) resulted in 13 compositions of EFT (Table 3.3). The data of drug dissolution have been fitted following Eq. (3.1) (Table 3.4) and/or Eq. (3.2) (Table 3.5).

In general the use of sodium bicarbonate (SB) in the above references was between 9–19% which is extended to 24% in this study to assure a durable matrix and a rapid floating of the tablet.

Compared to wet granulation (Basak et al. 2007), the optimization described here resulted in an analogous composition but solvents and lactose as excipients became redundant by the introduction of SA and melt granulation.

Table 3.5. Results from fitting the dissolution data following Eq. (3.2)

Tablet formulation	Release constant k_1	R^2
T1	0.434	0.997
T2	0.322	0.998
T3	0.381	0.989
T5	0.326	0.964
T7	0.343	0.999
T8	0.375	0.925
T9	0.429	0.994
T10	0.322	0.995
T11	0.334	0.991
T12	0.287	0.973

Table 3.6. Values of regression coefficients (R^2) and predicted residual sums of squares (PRESS) for different models

Responses (Y)	Models							
	Linear		Quadratic		Special Cubic		Cubic	
	R^2	PRESS	R^2	PRESS	R^2	PRESS	R^2	PRESS
H	0.963	54.39	0.978	31.62	0.980	27.47	0.980	18.86
FLT	0.805	809.23	0.922	311.88	0.924	296.53	0.957	129.34
$t_{60\%}$	0.867	334.00	0.889	263.00	0.889	256.00	0.954	63.00

For T4, T6, T13, Eq. (3.1) comprising Fickian diffusion and matrix erosion showed the best fit, and the dissolution profiles of the EFT were quite similar (Figure 3.1). For the most EFT, Eq. (3.2) based on Fickian diffusion only gave the best fit, and the dissolution profiles of these EFT were disparate (Figure 3.2). Since Fickian diffusion was the dominant dissolution mechanism, $t_{60\%}$ was below 4.38 h (Table 3.3). If the tablets resisted the erosion and, consequently, in this case, M molecules had to travel a bigger distance before being released into the surrounding liquid, $t_{60\%}$ was higher than 6.01 h up to 6.76 h (Table 3.3). Analyzing the domains of accepted values of response variables (Figure 3.3) one can draw the following conclusions:

- H is more influenced by the percentage of SA than by that of HPMC or SB, and the acceptable EFT hardness resulted for the minimum percentage of SA (12.8%). Also FLT is more influenced by the percentage of SA and SB than by that of HPMC, and there is a minimum percentage of SB (10.4%) and a maximum percentage of SA (17%) to accomplish acceptable values for both H and FLT at the same time.
- The domain of accepted values of $t_{60\%}$ is associated with SA percentages between 11.8% to 18.9% and percentages between 14.2% to 21.3% for both HPMC and SB.

3.3 Conclusion

We concluded that the optimized EFT contains between 15.6% and 24.2% HPMC, between 12.8 and 15.6% SA, and between 16.1% and 17.5% SB. In this study, SA, HPMC and SB (9–26%, 12–29% and 7– 24% successively) used percentages were roughly different from those that were utilized (0–2%, 17% and 10%successively) in the EFT of ranitidine hydrochloride (Dave et al. 2004). The authors have used low percentages of stearic acid (0–2%), and they applied chloroform to prepare the ranitidine hydrochloridestearic acid mixture. Considering the EFT

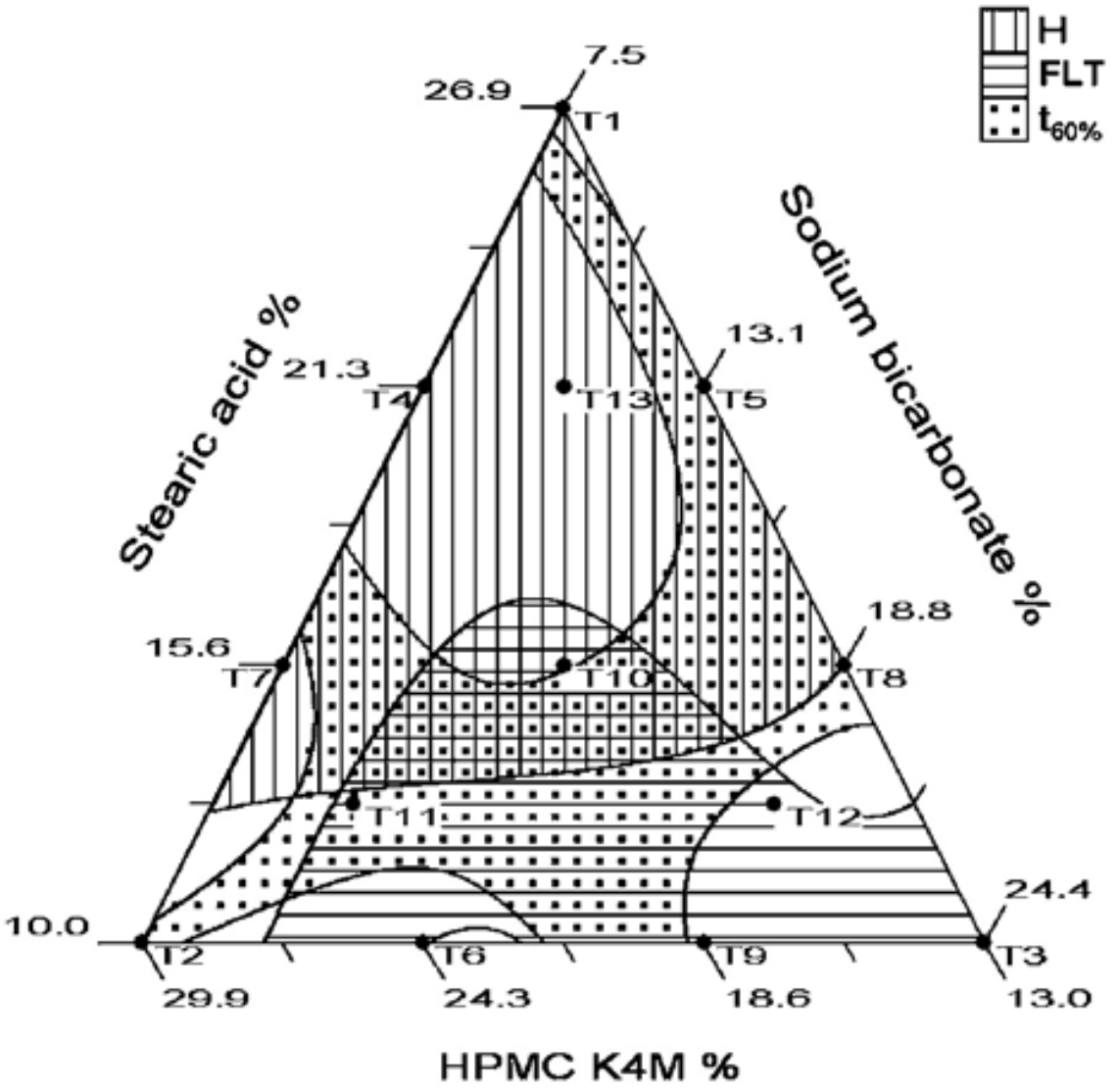

Fig. 3.3. Under constraints (Table 3.2) accepted values of H, FLT and $t_{60\%}$ as functions to percentages of SA, HPMC and SB.

containing carbamazepine (Patel et al. 2007) where the authors have used similar percentages of excipients to this study except beeswax instead of stearic acid, one can see nearly analogous dissolution rates between 6 and 8 h.

The use of hydrophobic components like SA, beeswax or compritol 888 in EFT did minimize the hydration rate of the matrix and also the variability of the dissolution profiles as shown for diltiazem hydrochloride (Gambhire et al. 2007). In addition, SA can enhance the physical properties of the tablets. H of the EFT selected by this study was greater than 12 kg/cm^2.

Percentages of HPMC equal to 20–22% were used in EFT of metformin hydrochloride (Basak et al. 2007) which were near the accepted range of HPMC resulting in the present study. Furthermore, it could be stated that the use of similar percentages of HPMC to prepare EFT resulted in $t_{60\%}$ near to 6 hours which was similar for dipyridamole (Patel and Patel 2007a,b) and for M as resulted from this investigation.

It must be noted that M and ranitidine hydrochloride are freely soluble in water. In contrast, dipyridamole and carbamazepine are poorly soluble in water. Nevertheless, similar percentages of HPMC have been used to prepare EFT, and the resulting dissolution profiles did not differ so much. Even domperidone have shown a good solubility in acidic pH but the use of 10–30% of HPMC resulted in drug dissolution of more than 10 h (Prajapati et al. 2008). On the other hand, high percentages (59–79%) of HPMC K4M as used in the preparation of EFT of atenolol, a drug that is sparingly soluble in water, resulted in drug dissolution of more than 60% nearby 6.5 h (Srivastava et al. 2005).

4 Optimization of the melt granulation process

4.1 Introduction

Fatty acids like stearic acid (SA) used in the melt granulation process of the metformin (M) tablets here are potentially suitable carriers for use in the design of drug delivery systems, being biocompatible, biodegradable, of low toxicity inexpensive, with drug release being approximately proportional to the Higuchi´s law of square root of time (Killen and Corrigan 2006). Özyazici et al. (2006) descript a suitable method to prepare slow release carriers based on higher temperatures where the fatty acid is molten. Metronidazole lipid matrix granules using several waxes and SA were prepared by this method and afterwards pressed to tablets.

We used melt granulation method to prepare M granules as precursor of the tablets based on hydroxypropyl methylcellulose.

To know if the melt granulation process parameters does influence the drug release. Two samples of granules were prepared using the same ratio SA:M but with different cooling techniques (Table 4.1).

Table 4.1. Metformin-stearic acid mixtures granules prepared by different cooling methods.

Sample	Cooling method	SA : M ratio	SA (g)	M (g)
i.31	Slow cooling	3:1	30	10
ii.31	Fast cooling	3:1	30	10

4.2 Results and discussions

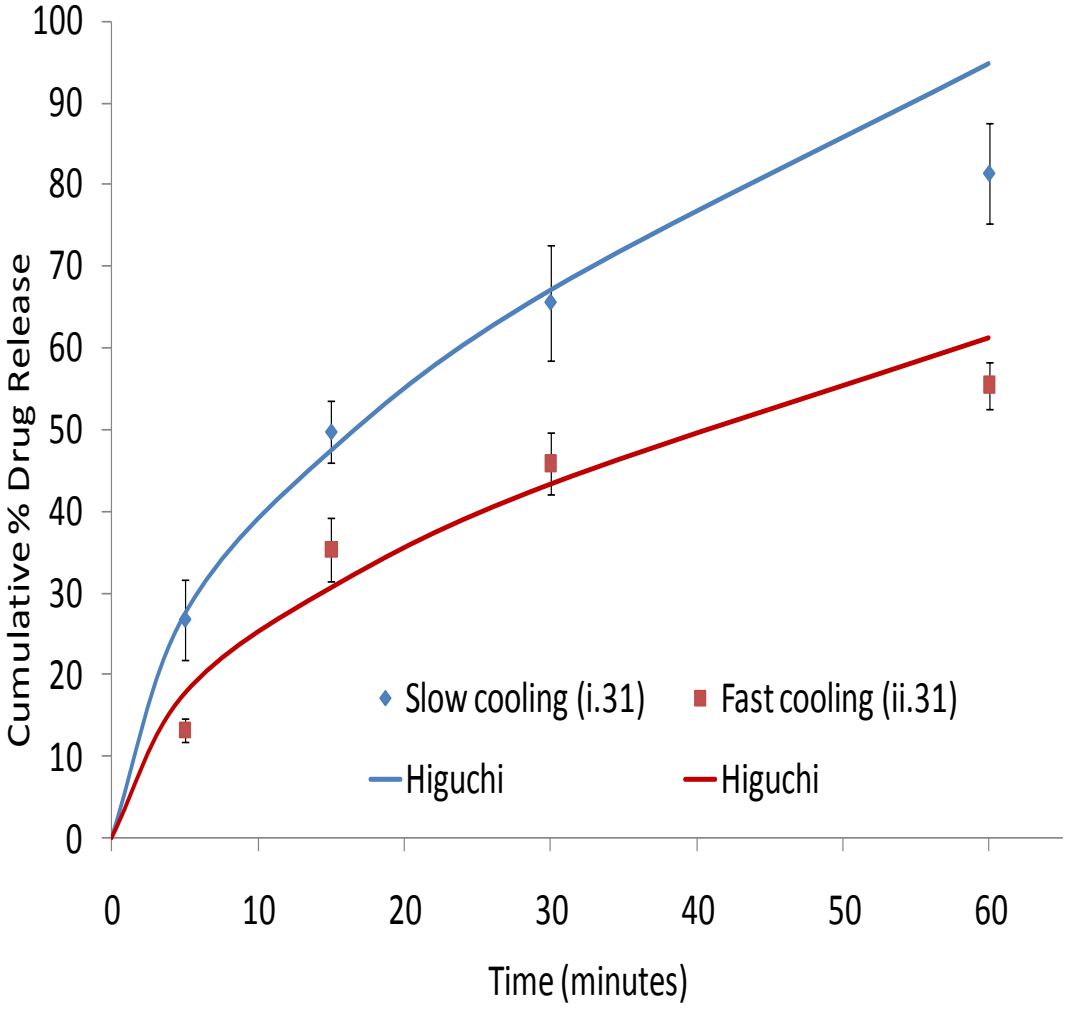

Figure 4.1. M release from granules samples i.31 and ii.31 (standard deviation marks for n=6). Only cumulative Drug release measurements <60% were fitted to Higuchi equation.

The drug release from the granules was found different for different cooling methods applied during granulate preparation (Figure 4.1) also the retardation of M release was stronger by shock cooling (Figure 4.1, ii.31 curve). To explain the release kinetic and transport mechanisms several commonly used equations for modeling release kinetics were applied (Dash et al. 2010). As shown Table 4.2, the sample i.31 and ii.31

were best fitted (its maximum Adj.R^2) to Higuchi model (Higuchi 1963) which is a diffusion controlled drug release throughout pores. Furthermore, Ritger et Peppas (1987) used a simple equation for description of solute release where k_p is a proportionality constant and n is the diffusion exponent. The k_p for both samples were significantly different (α=0.05), In contrast, n for both samples was near 0.5 which correspond to Fickian diffusion from thin film by anomalous transport mechanism (Serra et al. 2009).

4.3 Conclusion

To date there is no reference that shows interaction between SA and M (Bharate et al. 2010; Santos et al. 2008; Lerdkanchanaporn et al. 2001). In assumption of the absence of chemical interaction between M and SA and as was demonstrated using empirical mathematical models for drug release kinetics, the difference in drug release seen between different methods of cooling could be explained by difference in thickness and pores sizes of the thin film of SA that cover the small particles of M.
However, these models do not provide additional insights into transport mechanism. Furthermore, these models might fail whenever there is a need for taking into account specific physicochemical processes. Simply based on the diffusional exponent, n, of the Peppas models, can be misleading (Fu and Kao 2010).

Table 4.2. Fitting release results of M using empirical models for drug release kinetics*.

	Models								
	Zero Order $\frac{M_t}{M_\infty} = k_0 t$		First Order $\frac{M_t}{M_\infty} = 1 - e^{-k_f t}$		Higuchi $\frac{M_t}{M_\infty} = k_h \sqrt{t}$		Ritger -Peppas $\frac{M_t}{M_\infty} = k_p t^n$		
Samples	Adj. R^2	k_0†	Adj. R^2	k_f†	Adj. R^2	k_h†	Adj. R^2	k_p†	n
i.31	0.928	0.025	0.972	0.039	0.989	0.122	0.988	0.117	0.514
ii.31	0.892	0.011	0.948	0.016	0.984	0.079	0.973	0.057	0.597

* Only measurements M_t/M_∞ < 60% were considered, M_t and M_∞ are the amounts of drug released at time (t) and at equilibrium

†Significant difference between i.31 and ii.31 results (t – test, unequal variance, α=0.05, unequal sample size).

5 The combinatorial effects of polymers on the tablet performance

5.1 Introduction

Tamarind seed gum (TSG) was used by itself as an alternative for synthetic excipients as release retardant in tablet formulations (Deveswaran et al. 2009; Malviya et al. 2009; Sumathi and Alok 2002).

TSG is a polysaccharide quite familiar to other hydrophilic gums. These excipients were combined to increase viscosity which becomes greater if mixtures were used instead of the single components (Badillo and Ghaly 2008; Deshmukh et al. 2009; Salsa et al. 1997; Vincken et al. 1995). Hence different blends of TSG with semi synthetic polysaccharides as Hydroxypropyl methylcellulose (HPMC), sodium carboxy methylcellulose, ethylcellulose, cellulose acetate phthalate or with natural polysaccharides as gellan gum were used to prepare sustained release tablets (Kulkarni et al. 2008; Patel P et al. 2011). In addition, Patel B (2009) did investigate the use of TSG as mucoadhesive and sustained release component of nifedipine buccoadhesive tablet. The effect of several amounts of xanthan gum, HPMC, poly(vinyl pyrrolidone) (PVP) and other polymers to control the release rate was demonstrated for sustained release gastroretentive minimatrices containing amoxicillin trihydrate (Badhan et al. 2009). Recently, TSG with PVP were used to prepare metformin sustained release tablets (Sravani et al. 2012).

It is worthwhile; that formulating a floating mucoadhesive tablet of metformin (M) can be a challenging because of the drugs high solubility and the high drug content of the tablet (Rajab et al. 2010).

For gastro-retentive tablets, bioadhesion is often applied in combination with floating or swelling (Prinderre et al. 2011). TSG and its derivatives

were blended with HPMC and stearic acid (SA) to achieve M tablets with floating and mucoadhesive properties. To evaluate in vitro performance of the tablets described here adhesive and swelling properties were analyzed in parallel to drug release. To measure the adhesive properties, a method derived from Metia and Bandyopadhyay (2008) was adapted applying shear stress horizontally and resulting in work of adhesion. The work of tablet adhesion on stainless steel (W_{ss}) and the work of tablet adhesion on gastric mucosa (W_{gm}) were used to show the tablet adhesion properties.

In continuing this approach, PVP was introduced instead of hydrophobic SA as hydrophilic mucoadhesive component. The idea behind this approach was to take advantage of the conversion from bilateral to unilateral hydrogen bonding between HPMC polymer chains upon addition of PVP (Chan et al. 2003). The objectives of this study were

i. to investigate the in vitro performance of tablets containing HPMC as described earlier (Rajab et al. 2010) and tablets derived from this formula containing a fraction of TSG or its derivatives instead of HPMC. Two types of TSG derivatives were investigated, one carboxyl methyl ether (CMTG) and one hydroxypropyl ether (HPTG),

ii. to investigate combinatorial effects of PVP , TSG and HPMC on the in vitro performance of the more (hydrophilic) table using a mixture experimental design (Table 5.3),

iii. to compare tablets of HPMC and SA (Rajab et al. 2012a) with more hydrophilic tablets containing HPMC and PVP.

5.2 Results and discussions

Batches of formulations 0 to 9 (Table 5.1) and formulations T1 to T10 (Table 5.2) contained M within 100±5% of the labeled content (500 mg), and complied with pharmacopoeia specifications for weight variation (less than ±1%) and friability (less than 0.3%). The tablet hardness (H) was in range of 5 to 14 kg/cm^2. All tablets of different formulas floated (FLT) within 2 min and continued floating for 24 h.

As shown in the Table 5.1, W_{ss} of the HPMC tablets was 7.87 J/m^2 and the admixture of TSG or its derivatives mostly did not change significantly this basic value. Surprisingly two types of TSG decreased W_{ss} if they were embedded in a portion of 5%. HPTG decreased W_{ss} in a portion of 3.33%. These results suggested weaker adhesive power of TSG and its derivatives versus HPMC and were quite contrary to results of Patel et al. (2009) describing TSG as strongly bioadhesive. 60% of the metformin content was released within 3.81 h ($t_{60\%}$). No significant differences from this basic value of $t_{60\%}$ were noted for most of the formulas containing TSG or its derivatives. CMTG included in the formulas 1, 2, 3 increased $t_{60\%}$ significantly ($P < 0.02$), most if 3.33% CMTG was included as seen in formula 2. This effect might be based on higher viscosity of this derivative (5,500 to 11,000 mPa*s) slowing down drug release rate as observed by Tadros (2010) for HPMC and admixtures of alginates. Tablets of the formula 0 (HPMC only) achieved the 134% increase in tablet thickness after $t_{60\%}$ h (S). The admixture of TSG enhanced swelling considerably. S was found significantly higher ($P < 0.05$) for all tablets containing CMTG or FGTG. For the tablets containing HPTG, only the formula 5 showed a significant increase in S but this was clearly behind that of the formulas containing CMTG or FGTG. However, the swelling of HPMC tablets can be modulated by incorporating auxiliary additives into tablet formulations (Matharu et al. 2010), and TSG evoked this effect highly probable.

Table 5.1. W_{ss}, $t_{60\%}$ and S of the formulas 0 to 9 (tablets containing HPMC and SA).

No.	HPMC (%)	TSG code	Viscosity[1] (mPa*s)	TSG (%)	W_{ss} (J/m^2)	STD[2]	$t_{60\%}$ (hours)	STD[3]	S (%)	STD[2]
0	19.96	-	3,000 to 5,600	-	7.87	0.41	3.81	0.42	134.65	12.42
1	18.30	CMTG	5,500	1.66	8.56	0.55	5.17**	0.50	193.99*	12.59
2	16.63	CMTG	To	3.33	7.39	0.30	6.04**	0.30	210.07*	14.83
3	14.97	CMTG	11,000	4.99	5.87*	0.34	5.82**	0.29	210.91*	14.69
4	18.30	HPTG	5,100	1.66	7.71	0.80	3.79	0.29	162.20	12.78
5	16.63	HPTG	To	3.33	5.95*	0.53	3.70	0.24	173.18*	8.46
6	14.97	HPTG	6,300	4.99	7.06	0.23	3.47	0.27	156.85	13.76
7	18.30	FGTG	300	1.66	7.02	0.87	4.48	0.25	222.15*	11.98
8	16.63	FGTG	To	3.33	8.50	0.47	4.53	0.25	213.92*	12.10
9	14.97	FGTG	420	4.99	6.54*	0.05	4.40	0.25	215.40*	12.79

[1] 5% aqueous solution; STD[2], standard deviation (n=3); STD[3], standard deviation (n=6).

* significantly different from the corresponding result of formula 0 (α=0.05) .

** significantly different from the corresponding result of formula 0 (α=0.02).

Table 5.2. Composition of tablets (containing HPMC and PVP) and experimental results[a].

N	PVP (%)	TSG (%)	HPMC (%)	H (kg/cm^2)	SD	FLT (seconds)	SD	$t_{60\%}$* (hours)	SD	S* (%) (n=4)	SD	W_{ss} (J/m^2)	SD	W_{gm} (J/m^2)	SD
T1	10.3	0.0	20.6	5.60	0.19	28.50	0.50	2.19	0.32	264.56	31.52	6.72	0.13	4.07	0.15
T2	10.3	5.1	15.4	8.07	0.17	21.12	5.33	2.10	0.35	168.87	27.43	6.14	0.13	3.22	0.14
T3	15.4	0.0	15.4	10.58	0.10	15.17	0.90	1.71	0.55	140.18	31.20	4.84	0.10	2.21	0.11
T4	10.3	1.7	18.9	6.36	0.15	27.17	1.07	2.07	0.34	153.35	48.82	6.43	0.14	4.16	0.04
T5	12.0	0.0	18.9	6.99	0.10	27.50	1.50	1.85	0.40	152.98	42.63	5.82	0.23	3.67	0.13
T6	12.0	3.4	15.4	8.95	0.11	18.67	1.11	1.79	0.44	148.58	31.67	6.23	0.31	3.05	0.10
T7	10.3	3.4	17.2	7.13	0.15	24.83	1.67	2.05	0.35	162.73	34.97	6.10	0.11	3.86	0.16
T8	13.7	0.0	17.2	8.65	0.15	21.50	1.26	1.75	0.50	148.83	32.57	5.26	0.20	2.94	0.11
T9	13.7	1.7	15.4	9.84	0.17	17.50	1.61	1.75	0.51	143.87	33.17	5.68	0.18	2.92	0.11
T10	12.0	1.7	17.2	7.97	0.13	23.50	1.61	1.83	0.43	155.45	35.21	5.91	0.24	3.47	0.17

SD, standard deviation (n = 6)

[a]Every group of results, except groups with (*) superscript, did had 5 results at least who have significant difference in between them (t-test of difference, two tails, α=0.05).

Table 5.3. Factors, levels and responses in simplex lattice design.

Factors		Levels used				
	Compounds	Coded values	0	0.333	0.667	1
X_1	PVP		100.0	116.7	133.4	150.0
X_2	TSG	Actual values (mg)	0.0	16.7	33.4	50.0
X_3	HPMC		150.0	166.7	183.4	200.0
Responses						
Y_1	(Kg/cm^2)	Tablet hardness (H).				
Y_2	(seconds)	Tablet floating lag time (FLT).				
Y_3	(hours)	Time needed to release 60% of drug content ($t_{60\%}$).				
Y_4	(%)	Percent of tablet thickness after swelling for $t_{60\%}$ to it is original thickness (S).				
Y_5	(J/m^2)	Work of adhesion on stainless steel (W_{ss}).				
Y_6	(J/m^2)	Work of adhesion on rabbit gastric mucosa (W_{gm}).				

As shown in Table 5.2, H as a product specific parameter was found between 5.60 and 10.58 kg/cm^2, higher than 4.5 to 4.8 kg/cm^2 of sustained release matrix tablets of metformin (Dixit et al. 2009), and also higher than 3.5 to 7.5 found for a floatable gastroretentive tablet of metformin (Basak et al. 2007).

FLT was between 15.17 to 28.50 seconds, shorter than 15 to 42 seconds found for floatable gastroretentive tablets of metformin based on HPMC (Basak et al. 2007).

$t_{60\%}$ was calculated from dissolution results fitted to Eq. (5.1) as shown in Table 5.4 and it resulted between 1.71 to 2.19 hours. It was shorter than 1.9 to 6.8 for floated metformin effervescent floating tablet contained SA and HPMC (Rajab et al. 2010).

$$\frac{M_t}{M_\infty} = k_1 \times \sqrt{t} \qquad (5.1)$$

M_t/M_∞ is the percentage of drug released at time (t) and K_1 represents the Higuchi rate constant.

Table 5.4. Drug release results fitted according to

N	K_1 (%$h^{-0.5}$)	SE	R^2
T1	40.59	0.87	0.974
T2	41.42	0.98	0.968
T3	45.92	1.71	0.925
T4	41.68	0.95	0.970
T5	44.16	1.20	0.959
T6	44.82	1.33	0.951
T7	41.89	1.00	0.968
T8	45.42	1.53	0.937
T9	45.32	1.54	0.936
T10	44.35	1.30	0.952

SE, standard errors

S was calculated by applying Eq. (5.2) using $t_{60\%}$ from Table 5.2 and n_p with K_s shown in Table 5.5, where coefficients of determination R^2 for models fitting were significant (α = 0.05).

$S_t = K_s t^{n_p} + 100$ Where S_t is the swelling at time (t). (5.2)

S was between 140.18 and 264.56 % based on tablet thickness. This means a relative increase of the tablet thickness between 1.57 and 1.88 and that corresponds to a relative increase of tablet weight between 1.6 and 1.8 found for swelling of HPMC tablets after 15 min contact with water (Levina et al. 2007).

W_{ss} found between 4.84 and 6.72 J/m^2 was higher than W_{gm} ranging between 2.21 and 4.16 J/m^2.

Table 5.5. Swelling results fitted according to Eq. (5.2).

	n_p	SE	K_s (h^{-np})	SE	R^2
T1	0.63	0.01	100.35	0.46	0.997
T2	0.69	0.69	41.16	0.34	0.993
T3	0.79	0.79	26.32	0.30	0.988
T4	0.92	0.92	27.19	0.32	0.983
T5	0.87	0.87	31.16	0.33	0.987
T6	0.79	0.79	30.60	0.30	0.990
T7	0.81	0.81	35.02	0.31	0.991
T8	0.80	0.80	31.32	0.30	0.990
T9	0.80	0.80	27.95	0.30	0.988
T10	0.80	0.80	34.09	0.32	0.990

SE, standard errors

Combinatorial effects of PVP, TSG and HPMC

As displayed in Table 5.2 and as a result of the applied t-test, $t_{60\%}$ and S were not significantly influenced by the variation of polymer composition PVP, TSG and HPMC. That means swelling and drug dissolution were nearly independent from of hydrophilic polymers and mixture ratio. In contrary, H, FLT, W_{ss} and W_{gm} were found dependent on polymer composition. To show the combinatorial effects of the polymers and using

coded level of PVP (X_1), TSG (X_2) and HPMC (X_3) as parameters; experimental results of H (Y_1), FLT (Y_2) and W_{ss} (Y_5) were first fitted to quadratic model and W_{gm} (Y_6)was fitted to cubic model (Table 5.6). The fitted models were displayed as regression equations as in Table 5.7, to refine models, only statistically significant ($\alpha = 0.05$) coefficients were included in the equations. Positive coefficient of the factor in regression equation implies that the response increases with the factor. On the other hand, a negative coefficient represents a reciprocal relation between response and factor (Nutan et al. 2005).

As shown in Table 5.7, H (Y_1) was increased mostly when X_1 was increased and to lesser degree as response to the increase of X_2 and X_3 respectively.

FLT (Y_2) was decrease mostly when X_3 was decreased. Influence of X_2 was an average between X_1 and X_3.

W_{ss} (Y_5) was increased mostly when X_3 was increased alone. This was applied on X_2 with lesser degree.

More clearly, W_{gm} (Y_6) was increased when X_3 was increased. X_1 effect was at half of X_3.

The more than one factor term in regression equation signifies a non linear relation between response and the factors, hence the term considered as interaction term. A factor can produce different degree of effects on a response when used at different levels. Similar situation may arise when more than one factor are changed at the same time (Nutan et al. 2005). The equations were shown that interaction of X_1 with X_3 manifest a reciprocal effect on Y_1 and Y_5 while it had a cooperative effect on Y_2 and Y_6. The interaction of X_2 with X_3 displayed a reciprocal effect on Y_5 and a positive effect on Y_6. Furthermore, the interaction pattern of X_1 with X_2 was positive on Y_5 but on Y_6 it was influenced by the algebraic difference between X_1 and X_2.

Table 5.6. p-Values of sequential sum of squares model analysis and

	Sequential	**R^2**	**Adjusted R^2**	**Predicted R^2**
H				
Linear	<0.001	0.987	0.986	0.985
Quadratic	<0.001	0.992	0.991	0.990
Special	0.677	0.992	0.991	0.989
Cubic	0.864	0.992	0.990	0.988
FLT				
Linear	<0.001	0.764	0.756	0.730
Quadriatic	0.020	0.803	0.785	0.743
Special	0.997	0.803	0.781	0.737
Cubic	0.643	0.810	0.776	0.726
W_{ss}				
Linear	<0.001	0.754	0.746	0.730
Quadratic	<0.001	0.896	0.886	0.875
Special	0.806	0.896	0.884	0.868
Cubic	0.390	0.902	0.884	0.859
W_{gm}				
Linear	<0.001	0.896	0.893	0.883
Quadratic	<0.001	0.950	0.945	0.939
Special	0.135	0.952	0.947	0.938
Cubic	0.034	0.960	0.953	0.942
Tested				
Linear	$Y= b_1X_1 +b_2X_2 + b_3X_3$			
Quadratic	$Y= b_1X_1 +b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$			
Special cubic	$Y= b_1X_1 +b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3$			
Cubic	$Y= b_1X_1 +b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{123}X_1X_2X_3 + c_{12}X_1X_2(X_1 - X_2) + c_{13}X_1X_3(X_1 - X_3) + c_{23}X_2X_3(X_2 - X_3)$			

Table 5.7. Regression equations of the fitted models[a]

$Y_1 = 10.64X_1 + 8.05X_2 + 5.56X_3 - 1.23X_1X_3$
$Y_2 = 14.99X_1 + 21.53X_2 + 29.28X_3 + 11.03X_1X_3$
$Y_5 = 4.83X_1 + 6.14X_2 + 6.73X_3 + 2.10X_1X_2 - 1.11X_1X_3 - 0.79X_2X_3$
$Y_6 = 2.21X_1 + 3.23X_2 + 4.09X_3 + 1.12X_1X_2 + 0.63X_1X_3 + 1.48X_2X_3 + 1.43X_1X_2(X_1 - X_2)$

[a]Only the terms with statistical significance are included.

As shown in Table 5.8, the small Standardized main effect (SME) of interactions terms suggests that they had weak importance in predicting responses. In the present study, R^2 values (>79%) represent a reliability of the design. Besides, the p-Value for lack of fit for all models were greater than 0.05, suggesting absence of any lack of fit of the models and that also strengthened the reliability of the models.

Comparison of tablets containing HPMC and SA with tablets containing PVP

As shown in Table 5.9, H of tablets containing PVP was found significantly larger; FLT and $t_{60\%}$ were found significantly shorter than for tablets containing HPMC and SA reported earlier by Rajab (2012a). Differences in S between tablets with PVP versus tablets with HPMC and SA were non-significant. Differences in W_{ss} were significant except when TSG was applied in the lowest level. In general, W_{ss} was higher for tablets containing HPMC and SA than for tablets with PVP, and W_{gm} was found significantly higher for tablets with PVP than for tablets containing HPMC and SA.

Table 5.8. Standardized main effects of the factors on the responses[a]

	Standardized main effect (SME)			
	Y_1	Y_2	Y_5	Y_6
X_1	219.54	20.14	66.82	42.84
X_2	188.25	32.77	84.93	62.76
X_3	114.79	39.35	93.05	81.50
X_1X_2	-	-	6.56	5.04
X_1X_3	-5.07	2.97	-3.47	2.82
X_2X_3	-	-	-2.45	6.62
$X_1X_2(X_1 - X_2)$	-	-	-	2.67
R^2	99.09%	79.66%	89.56%	95.60%
p-Value of lack of fit	0.439	0.748	0.542	0.216

[a]Only the terms with statistical significance are included.

Table 5.9. Comparison of tablets containing HPMC and SA (Rajab et al. 2012a) with tablets containing HPMC and PVP.

TSG (%)	HPMC (%)	PVP (%)	No.	H (kg/cm^2)	SD	FLT (seconds)	SD	$t_{60\%}$ (hours)	SD	S (%)	SD	W_{ss} (J/m^2)	SD	W_{gm} (J/m^2)	SD
1.7	18.9	+	T4	6.36	0.15	27.17	1.07	2.07	0.34	214.89 [a]	10.75	6.43 [d]	0.14	4.16	0.04
1.7	18.3	-	7	2.75	0.40	40.33	1.70	4.48	0.25	222.15 [a]	11.98	7.02 [d]	0.87	1.42	0.12
3.4	17.2	+	T7	7.13	0.15	24.83	1.67	2.05	0.35	212.26 [b]	11.58	6.10	0.11	3.86	0.16
3.3	16.6	-	8	3.31	0.37	32.50	1.50	4.53	0.25	213.92 [b]	12.10	8.50	0.47	1.50	0.13
5.1	15.4	+	T2	8.07	0.17	21.12	5.33	2.10	0.35	217.12 [c]	13.54	6.14	0.13	3.22	0.14
5.0	14.9	-	9	3.71	0.11	44.20	2.23	4.40	0.25	215.40 [c]	12.79	6.54	0.05	1.10	0.16

+ Tablets containing HPMC and PVP (without SA); -, Tablets containing HPMC and SA (without PVP).

[a], [b], [c], [d] Unequal variance and unequal sample size t-test (two tails, α=0.05) showed no significant difference between those pair of results.

5.3 Conclusion

The use of a fraction of TSG or its derivatives instead of HPMC in the tablets can help to adjust swelling and M release whilst adhesion of the swollen tablets remains unchanged.

The different in vitro performance of tablets containing different water soluble polymers could be explained partially by the differences in the hydrophilic properties of the polymers. Some interactions revealed in the present work such as the interaction between PVP and HPMC was previously documented by Chan et al. (2003) and applied in mucoadhesive minimatrices by Karavas et al. (2006). However, there is a rest of uncertainty that needs further investigations especially the interaction between TSG and HPMC or PVP.

6 Relationship between adhesion on steel and mucoadhesion of the tablets

6.1 Introduction

Despite the intense focus surrounding mucoadhesive systems over the past few decades, to date bioadhesive polymers have not achieved clinically significantly improved gastric retention time (Laulicht et al. 2009). In addition, no standard test methods have been specifically designed for mucoadhesion analysis. This places limitations upon the direct comparison of data obtained from different research groups (Andrews et al. 2009). Nevertheless, three main testing strength modes are recognized – tensile, shear, and peel stresses.

The most popular technique used to measure force of separation in bioadhesive testing is the application of force perpendicularly to the tissue/adhesive interface, during which a state of tensile stress is set up. But during the shear stress, the direction of the forces is reoriented so that it acts along the joint interface. The peel test is based on the calculation of energy required to detach the patch from the substrate (Shaikh et al. 2011).

Due to differences between the in vitro screening conditions and the in vivo bioadhesive environment, Laulicht et al. (2009) did not find in vivo and in vitro correlation for polyanhydride polymers tensile stress measurements obtained from live rat stomach and excised tissue of rat stomach.

To overcome on high variability of tensile stress measurements, Pund et al. (2011) studied the combinatorial effects of Carbopol and Avicel using the work of tensile stress need it for rifampicin tablet to be detached from porcine gastric mucosa. Later, he assessed the gastric retention time of the optimized tablet by using gamma scintigraphy in human.

As mentioned above, a standard method of adhesion is necessary to be able to compare results of different research group, to fast screen for adhesion material and to be used as reliable reference for further development. The variability between different animal gastric mucosa impose the use of substrate made from standard and well characterized materials like stainless steel, porcelain, glass, plastic, fiber texture…etc where each substrate could reveal different adhesion mechanisms. The strength measured by this method is the shear stress, which is, in contrary to tensile stress, it mimics the movement of tablet on the surface of gastric membrane. To enhance the reproducibility, the work of adhesion of shear stress is calculated.

The objectives were to

- measure the work of adhesion of on stainless steel (W_{ss}) plate for 10 different tablet formulations,

- measure the work of muccoadhesion on rabbit gastric mucosa (W_{gm}) for the same tablet and

- investigate of the relation between results of both methods.

6.2 Results and discussions

Table 6.1. Composition of tablets and experimental results.

N	PVP (%)	TSG (%)	HPMC (%)	W_{ss} (J/m^2)	SD	W_{gm} (J/m^2)	SD
T1	10.3	0.0	20.6	6.72	0.13	4.07	0.15
T2	10.3	5.1	15.4	6.14	0.13	3.22	0.14
T3	15.4	0.0	15.4	4.84	0.10	2.21	0.11
T4	10.3	1.7	18.9	6.43	0.14	4.16	0.04
T5	12.0	0.0	18.9	5.82	0.23	3.67	0.13
T6	12.0	3.4	15.4	6.23	0.31	3.05	0.10
T7	10.3	3.4	17.2	6.10	0.11	3.86	0.16
T8	13.7	0.0	17.2	5.26	0.20	2.94	0.11
T9	13.7	1.7	15.4	5.68	0.18	2.92	0.11
T10	12.0	1.7	17.2	5.91	0.24	3.47	0.17

SD, standard deviation (n = 6)

To check the correlation between W_{ss} and W_{gm} Spearman's rank correlation coefficient was calculated using results shown in Table 6.1. It was equal to 0.770 (two tails, α = 0.05) which means that the rank order obtained for adhesion on stainless steel was in agreement with the rank order obtained for the mucoadhesion, furthermore, when W_{gm} regressed on W_{ss} in a simple linear model the coefficient of determination R^2 was equal to 0.705 (α = 0.05), the slope was 0.92 (α = 0.05) and the intercept was -2.09 J/m^2 (p = 0.133), Eq. (6.1).

$$W_{gm} = 0.92W_{ss} - 2.09 \qquad (6.1)$$

This result corresponds to investigations showing the rank order of various polymers regarding time of adhesion of compressed discs to the porcine small intestinal mucosa with the rank order obtained for total

work of adhesion (Grabovac et al. 2005). The moderated value of R^2 could be explained by the influence of mixture compounds on W_{ss} and W_{gm}

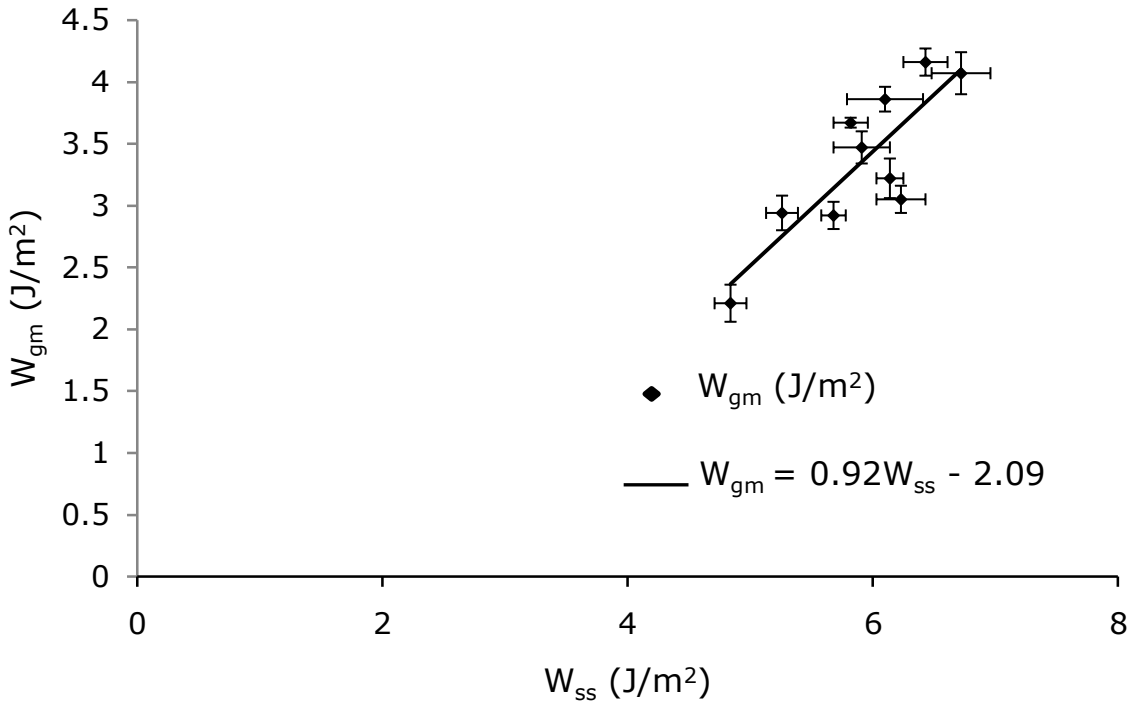

Figure 6.1. Simple linear regression between W_{gm} and W_{ss} (n=6).

differences that were evident from the three-dimensional response surface plots (Nutan et al. 2005; Pattnaik et al. 2011) where at lowest level of HPMC shown in left side plans of Figures 6.2 and 6.3, the response surface of W_{ss} had a positive curvature which corresponded to a positive interaction between PVP and TSG but the response surface of W_{gm} was positively curved near high levels of PVP and it was slightly negatively curved at low levels of PVP which corresponded to an interaction between PVP and TSG influenced by the difference between them. As shown in both figures, surfaces were flattened and raised gradually with increased levels of HPMC. This could explain the agreement in the rank order between W_{ss} and W_{gm}.

6.3 Conclusion

Measurement of adhesion on stainless steel was simpler feasible than measurement of mucoadhesion. The results of both measurements were found correlating and therefore the equation (6.1) is usable to conclude mucoadhesion based on adhesion measurements on stainless steel.

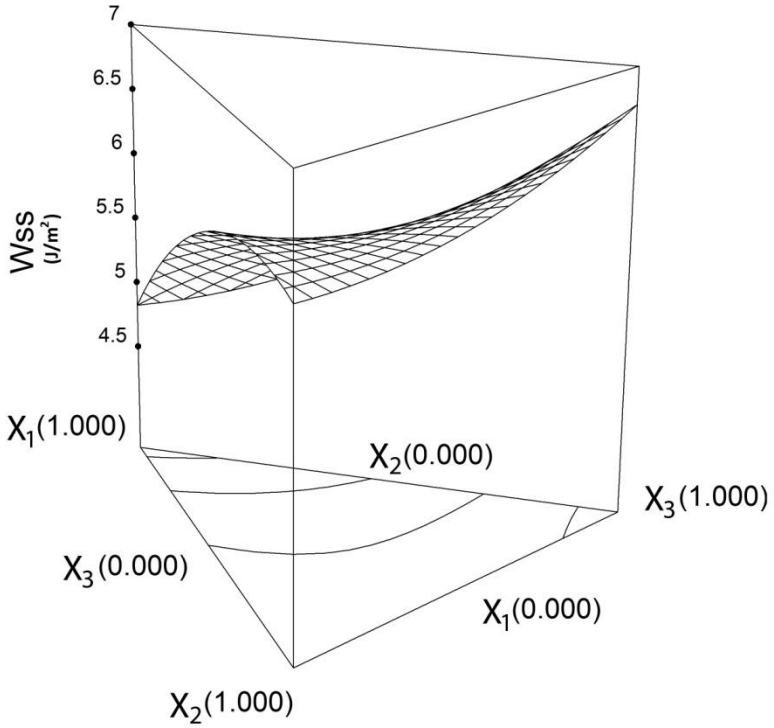

Figure 6.2. Three-dimensional response surface plot for W_{ss} indicating the effects of the mixture components.

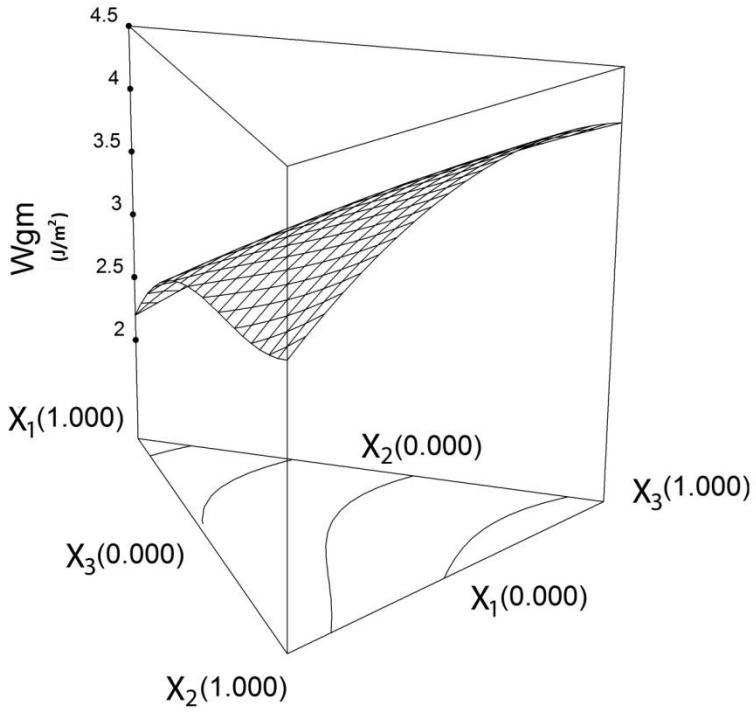

Figure 6.3. Three-dimensional response surface plot for W_{gm} indicating the effects of the mixture components.

7 Selection of the new dosage form, discussion and conclusion

The working hypothesis of this work was to develop a tablet of metformin that realizes drug release according to the most modern biologic findings, which means drug release that mimics the circadian variation of glucose levels.

This hypothesis resulted from knowledge that an optimal therapy of diabetic disease using metformin alone or in combination with other oral anti diabetic should consider the circadian rhythm. In our investigations to confirm this idea, we did adapt gastric retentive tablet that uses the effervescent floating principle. We did notice that introducing stearic acid using melting process can enhance the physical properties of the tablet. Also, the optimization of this process revealed the possibility to use stearic acid as release controller of metformin from hydrophilic matrix.

From these investigations a tablet resulted consisting of metformin 13.4% stearic acid, 20.0% Hydroxypropyl methylcellulose (HPMC), 14.0% sodium bicarbonate, 2.0% citric acid, 0.4% magnesium stearate and 0.4% Aerosil. This tablet was prepared using melt granulation and was characterized by in vitro performances as shown in the Table 7.1.

Table 7.1. In vitro performance of optimized floating gastroretentive tablet and the target properties aimed for optimal therapy.

In vitro performances (unit)	Target properties	Tablet properties	Target achievment
Tablet (diameter)X(thickness) (mm)	Smaller is better	(16 ± 0.1) X (3.5 ± 0.4)	Not achieved
Friability (%)	0.08 to 0.15	0.10 ± 0.01	Achieved
Hardness (kg/cm^2)	4 to 12	5.16 ± 0.28	Achieved
Floating lag time (minutes)	Less than 3	2.00 ± 0.37	Achieved
Time to dissolve 60% of drug ($t_{60\%}$, hours)	3 to 4.5	3.81 ± 0.42	Achieved
Swelling at $t_{60\%}$ (% to initial diameter)	Non specified	134.65 ± 12.42	
Adhesion on stainless steel* (J/m^2)	5 to 10	7.87 ± 0.41	Achieved
Mucoadhesion on gastric rabbit mucosa* (J/m^2)	2 to 5	1.42 ± 0.12	Not achieved
Stability tests 25°C, 60%RH	Conform USP 32	Conform USP 32	Achieved

* Estimated after 1 hour of swelling as the work of adhesion of shear stress during 3 minutes (J) devised on tablet surface (m^2).

Furthermore, the use of a fraction of tamarind seed gum (TSG) or its derivatives instead of HPMC in the tablets can help to adjust swelling and metformin release whilst adhesion of the swollen tablets remains unchanged. This finding oriented our work to investigate the combinatorial effects of TSG and HPMC using a more soluble polymer like poly(vinyl pyrrolidone) (PVP) and to measure their influences on in vitro

performance. As result, the interaction between HPMC and PVP, previously documented by Chan et al. (2003), was noted beside a possibility of interaction between TSG and HPMC or PVP. From these investigations where we used two different method of preparation we concluded that TSG was not able to enhance the adhesion of the tablet.

To measure adhesion or mucoadhesion we developed an apparatus that was able to measure the work of adhesion of the shear stress. Results of using this apparatus in investigations carried on tablet prepared with PVP were significant to predict a moderated relationship between adhesion on stainless steel and mucoadhesion measurements. While the absence of a standard method for adhesion in pharmaceutical domain renders those results incomparable with other works yet it could be a first step to develop in-house (Martin-Luther University and Arabic International University) adhesion standards materials and method.

The results of the in vitro performance of the table were partially confirmed by a pilot biostudy on human subjects that was carried on to compare its pharmacokinetics parameters with leader market drug. This study gave evidence that using simple and inexpensive materials and methods of preparation could obtain verities of oral controlled dosage forms with in vivo performances comparable to a more complex and expensive ones.

In further elaboration, we were been able to develop and to optimize a two-pulse releasing tablet for once daily use of anti-diabetic drugs like metformin. The tablet provides two peaks in the blood plasma concentration versus time profiles that mimic the desirable biologic rhythms.

This leads to an invention of a new oral dosage form (Rajab et al. 2012c). The inventive formulation increases therapeutic efficacy of the drug and improves patient compliance due to once daily administration and reduced side effects (Table 7.2).

Table 7.2. Facts/parameters of some metformin products in market and the new oral dosage form.

Facts/Parameters	Products in market	New oral dosage form
Drug release characteristics (Drug)	Immediate-release (Glucophage®) Gastroretentive-release (Glumetza®) Extended-release (Fortamet®)	Two-pulse -release and gastro- retentive.
Costs for one tablet ($)	0.3[1] to 4.5 (branded) for 750 mg tablet	0.07[2] for 500 mg tablet.
Dose	High (1.5 - 2.0 g/day)[3]	Low (1.0 g/day)
Bioavailability	Low[3](60%)	High[4] (>75%)
Lowering effect of glucose in plasma	Immediate release: treatment diverted by patient unawareness. Extended release: constant metformin concentration even with glucose is low.	Better lowering glucose effect: the metformin concentrations follow the glucose levels in plasma. (to be proved by future clinical study)
Gastro-intestinal side effects (diarrhea, nausea, vomiting…etc)	30%[3]	< 15% expected on basis of drug local gastroretentive release and enhanced intestinal absorption.

[1] Consumer costs (Jabbour and Zing 2011).

[2] Bulk ware ex factory NCPI calculated on basis of economical batch size.

[3] The incidence of associated gastrointestinal side effects with the use of metformin immediate release usually increases with increasing dose (Bouchoucha et al. 2011; Jabbour and Zing 2011).

[4] Study report (Arafat, 2011).

8 Biostudy on human volunteers

8.1 Introduction

The assessment of the products included in this study was designed so that an open label, single dose of the Diaphage® GR 500 mg tablet (test) was compared with the Glucophage® XR 750 mg tablet (reference) in healthy male subjects.

6 Healthy male, aged 18 to 50 years, subjects satisfied the inclusion criteria and checked for specific exclusion criteria were hospitalized the night before the start of the study and they received a standard supper before 7.30 p.m. and they fasted overnight for ten hours, and given a single oral dose of either test or reference drug. A blood sample was taken before subjects having drug samples, then after post – dosing blood samples were drawn at scheduled time intervals. A standard breakfast and a standard lunch were offered to all subjects 4 and 9 hours respectively after drug intake. The subjects were supervised throughout the period of the study.

The objectives of this study were to obtain the pharmacokinetics of Diaphage® GR 500 mg tablet and Glucophage® XR 750 mg, and to compare between both products.

8.2 Results and discussions

Tablets with composition described in chapter 7 and directed to achieve bioequivalence to the marketed product (Glucophage® XR 750 mg) were manufactured by the procedure described here (9.2.2) and by using the pilot plan equipments of National Company for Pharmaceutical Industry (NCPI), Aleppo, Syria. The results of the optimized tablet showing the

mentioned two peaks profile could not be presented here to cover the know-how of the invention announced to be patented (Rajab et al. 2012c).

6 Healthy male were enrolled in two-way single-dose crossover study, there were no withdrawals and all of them completed the study. No side effects were reported during the study period. Individual plasma concentrations of metformin (ng/ml) following the administration of test and reference tablets (Tables 8.1; 8.2) were used to calculated metformin pharmacokinetic parameters (Table 8.3) by using non compartmental approach (Table 8.4).

In addition, results were fitted to one compartment model for a sustained release formulation (Wiegand and Taylor 1960) described in Table 8.5. Means of calculated parameters (Table 8.6) were used to draw curves (Figure 8.1; 8.2) and to compare between both products.

Results of non compartmental approach

As shown in table 8.3, the dose strength ratio between test and reference drug was 500/750 (66.67%) while C_{max} ratio was 607.881/712.015 (85.37%) and AUC ratio was 3424.520/4211.175 (81.32%) for AUC_{0-t} and 3906.278 /5150.680 (75.8%) for $AUC_{0-\infty}$. Hence the rate and the extent of absorption of metformin as expressed by C_{max} and AUC respectively of test drug were higher than that of the reference drug.

Considering using Diaphage® GR 500 mg or Glucophage® XR 750 mg treatments as the only source of variation the difference between averages values was not significant for C_{max}, yet it was significant for AUC_{0-t}. (ANOVA two way test, α=0.05).

Table 8.1. Individual plasma concentrations of metformin (ng/ml) following the administration of Diaphage® GR 500 mg tablet (test).

Time (hours)	1	2	3	4	5	6	Average	STD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	0.00	0.00	0.00	56.32	199.03	57.99	52.22	77.18
1	208.53	100.63	201.18	180.61	425.11	346.83	243.82	119.20
1.5	175.81	152.94	194.02	79.80	353.98	136.28	182.14	92.89
2	304.29	180.85	652.59	182.50	481.34	160.40	327.00	200.17
2.5	275.33	**366.93***	504.08	218.52	382.16	231.26	329.71	109.14
3	**522.96***	319.00	**826.17***	625.72	461.78	**368.30***	520.65	185.33
4	221.14	279.67	812.22	**718.61***	**843.90***	332.06	534.60	286.66
5	279.25	364.01	433.28	659.87	472.93	319.82	421.53	136.74
6	212.37	303.82	399.16	657.92	401.69	267.52	373.75	157.73
8	163.17	215.99	259.19	353.33	289.74	178.69	243.35	71.91
10	190.33	157.38	284.90	224.03	142.15	89.59	181.40	68.05
12	44.33	113.46	94.09	143.66	104.74	101.79	100.34	32.39

* C_{max} maximal concentration after tablet administration.

Table 8.2. Individual plasma concentrations of metformin (ng/ml) following the administration of Glucophage® XR 750 mg tablet (reference).

Time (hours)	1	2	3	4	5	6	Average	STD
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	25.04	30.61	148.89	40.84	52.64	89.60	64.61	47.24
1	193.36	276.87	186.21	452.51	247.47	363.15	286.59	103.71
1.5	411.16	347.37	375.74	449.15	346.91	**412.93***	390.54	40.86
2	**522.77***	618.45	430.25	514.96	545.63	325.54	492.93	101.84
2.5	413.21	**678.99***	398.64	433.90	625.96	338.06	481.46	137.29
3	519.69	362.01	528.64	590.87	440.67	393.38	472.54	88.17
4	486.82	353.24	545.20	**851.31***	688.45	333.05	543.01	199.73
5	256.66	185.71	**731.15***	768.25	**1074.95***	214.30	538.50	370.59
6	220.18	170.84	620.01	603.14	636.00	172.49	403.78	237.44
8	136.14	99.59	383.44	592.61	588.54	231.08	338.56	218.48
10	140.31	146.23	248.16	459.66	252.20	188.22	239.13	118.22
12	64.28	8.33	226.84	155.18	228.57	253.60	156.13	100.03

* C_{max} maximal concentration after tablet administration.

Table 8.3. Metformin pharmacokinetic parameters of Diaphage® GR tablets and Glucophage® XR .

Drug (test/reference)	Dose (mg)	C_{max} (SD) (ng/ml)	T_{max} (SD) (hours)	AUC_{0-t} (SD) (ng.hour/mL)	$AUC_{0-\infty}$ (SD) (ng.hour/mL)	$T_{1/2}$ (SD) (hours)	K_{el} (SD) ($hours^{-1}$)
Diaphage® GR (test)	500	607.81 (218.30)	3.25 (0.61)	3424.52 (1028.59)	3906.28 (1056.32)	3.32 (0.55)	0.21 (0.03)
Glucophage® XR (reference)	750	712.02 (235.73)	3.33 (1.54)	4211.18 (1623.91)	5150.68 (2097.10)	3.21 (0.82)	0.23 (0.08)

Table 8.4. Non compartmental approach (Housman et al. 2012).

Parameters	Definition	Method of estimation
C_{max}	Maximal concentration after administration.	Observed from Table 8.1 or 8.2.
T_{max}	Time to reach C_{max}.	Observed from Table 8.1 or 8.2.
C_t	Last measurable concentration.	Observed from Table 8.1 or 8.2.
AUC_{0-t}	Area under the concentration - time curve (time 0 h to C_t) drawn using Table 8.1 or 8.2.	Calculated by the trapezoidal rule for curves.
$AUC_{0-\infty}$	The area under the plasma concentration versus time curve from time (0) to infinity.	$AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el}$
$T_{1/2}$	Terminal half –life.	$T_{1/2} = Ln(2/K_{el})$
K_{el}	Apparent first-order elimination or terminal rate constant.	Last three (or more) non-zero plasma concentrations, C(t), (Figures 8.1) were fitted using least-squares method to the equation: $C(t) = A \times e^{-K_{el}t}$ (8.1)

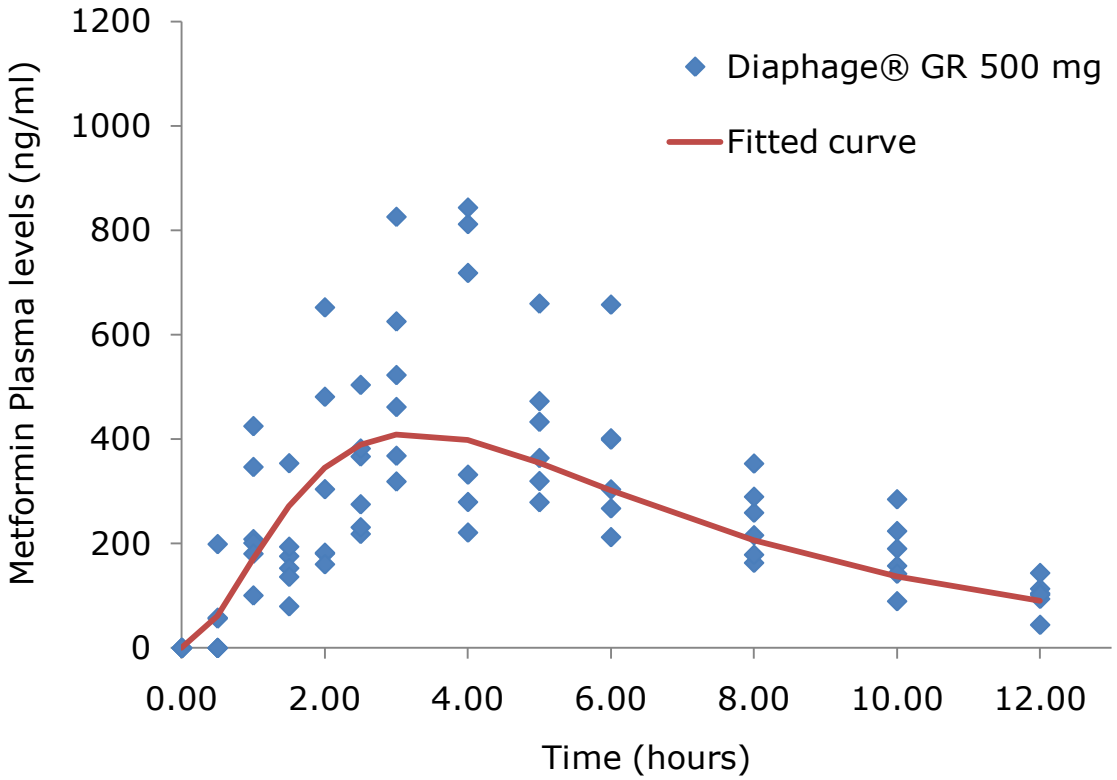

Figure 8.1. Metformin plasma concentration-time results, curve fitted to compartmental model equation (8.2). Administration of Diaphage® GR 500 mg tablet was done under fasting conditions to 6 males.

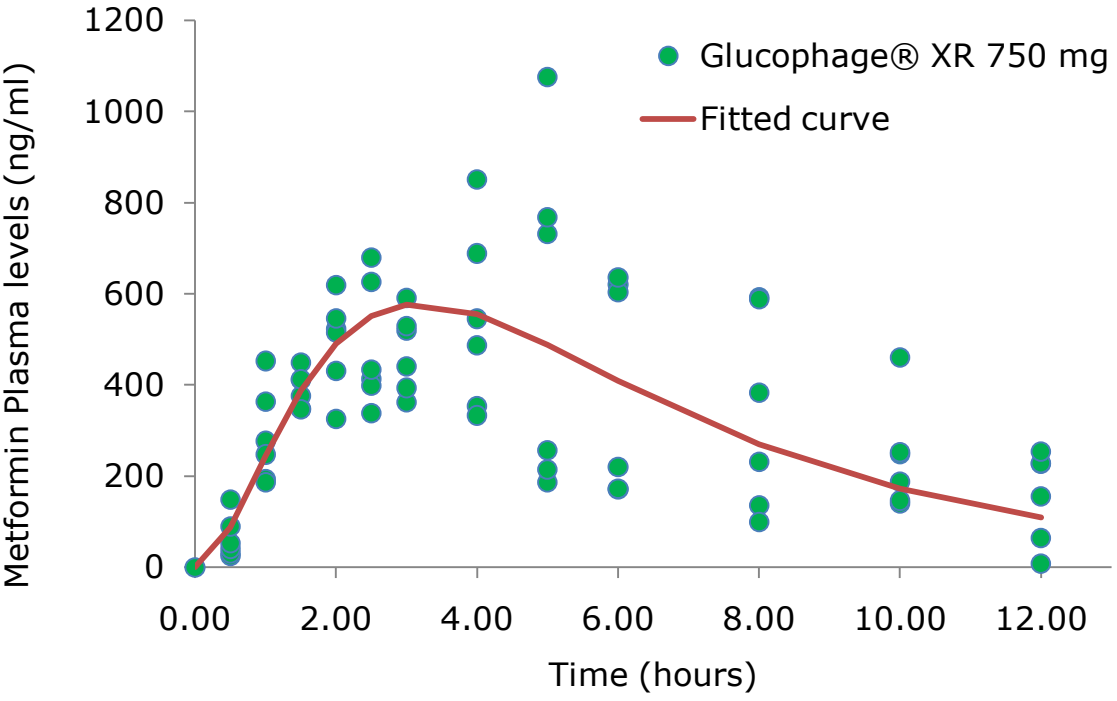

Figure 8.2. Metformin plasma concentration-time results, curve fitted to compartmental model Eq. (8.2). Administration of Glucophage® XR 750 mg tablet was done under fasting conditions to 6 males.

Table 8.5. One compartment model for a sustained release formulation (Wiegand and Taylor 1960).

Model equation:

$$C(t) = \frac{SD_0 f_r k_a k_r}{V_d(k_a - k_r)(k_e - k_r)}(e^{-k_r t} - e^{-k_e t}) + \frac{SD_0 f_i k_a - \left(\frac{SD_0 f_r k_a k_r}{(k_a - k_r)}\right)}{V_d(k_e - k_a)}(e^{-k_a t} - e^{-k_e t}) \quad (8.2)$$

Parameters	Definition
C(t)	Plasma concentration metformin (mg/L)
S	Salt factor = Metformin molecular weight / metformin HCl molecular weight= 0.78.
D_0	Labeled dose (mg). D_0=500 mg for Diaphage® GR and D_0=750 mg for Glucophage® XR.
V_d	Apparent volume of distribution (L).
k_e	Elimination rate constant (h^{-1}) equal to K_{el}.
k_a	Absorption rate constant (h^{-1}).
k_r	Release rate constant (h^{-1}).
f_r	Fraction of drug released with first order kinetics.
f_i	Fraction of drug available immediately, where $f_i+f_r=1$.

Table 8.6. Pharmacokinetics and drug release parameters resulted from fitting to one compartment model for a sustained release formulation (Table 8.5).

Diaphage® GR 500 mg								
Const.	1	2	3	4	5	6	Mean	STD
k_e	0.23	0.16	0.21	0.25	0.24	0.19	0.21	0.03
V_d	670	750	400	390	385	755	558.33	168.98
k_a	1.05	0.90	1.20	0.70	1.30	0.80	0.99	0.21
k_r	1.20	1.20	1.05	0.60	0.80	1.20	1.01	0.23
f_r	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
f_i	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R^2	0.911	0.961	0.914	0.865	0.918	0.892	0.819*	
Glucophage® XR 750 mg								
Const.	1	2	3	4	5	6	Mean	STD
k_e	0.18	0.36	0.17	0.22	0.22	**	0.23	0.08
V_d	850	590	630	400	400		574.00	187.16
k_a	1.06	1.20	1.20	0.90	0.90		1.05	0.15
k_r	2.00	1.10	0.60	0.50	0.50		0.94	0.64
f_r	1.00	1.00	1.00	1.00	1.00		1.00	0.00
f_i	0.00	0.00	0.00	0.00	0.00		0.00	0.00
R^2	0.956	0.930	0.961	0.917	0.940		0.849*	0.08

* resulted from fitting M plasma levels to the Eq. (8.2) using means.

** Unable to evaluate K_{el}

Results of one compartment model

As explained in Table 8.5, M in plasma levels were fitted to one compartment model for a sustained release formulation using non linear optimization method implemented in MS Excel. Except for salt factor S that was constant, the labeled dose D_0 that was different between the two products and the elimination rate k_e that was considered equal to K_{el} of each product for each volunteer, all other pharmacokinetics and drug release parameters were variables in the fitting process. The only constraint was the sum of f_i and f_r must equal to one.

As shown in Table 8.6, result of apparent volumes of distribution (V_d) of M were in range between 385-850L as was reported by Graham et al. (2011). All pharmacokinetics and drug release constants did not show any significant difference between the two drugs (t-test, unequal sample sizes, unequal variance, α=0.05).

As shown in Figure 8.1 and 8.2, plasma concentration-time curves were displayed by applying means, reported in Table 8.6, in model for a sustained release formulation. As expected from the results of fitting, it can be seen that the two curves are parallel to each other nevertheless they present different doses of M.

8.3 Conclusion

The present study shows that Diaphage®GR 500 mg could have better bioavailability than the reference drug Glucophage®XR 750 mg. Nevertheless, this study was done on 6 healthy subjects under fast condition hence it needs to be confirmed by a larger study and in fed conditions.

9 Materials and methods

9.1 Materials

Metformin hydrochloride (M) (Ph. Eur. quality) supplied by Abhilash Chemicals Pvt. Ltd, Madurai, India; Hydroxypropyl methylcellulose (HPMC, Methocel K4 M) was from Dow Europe GmbH, Stade, Germany; highly purified food grade tamarind seed gum (TSG, TA400, CAS : 39386-78-2) carboxymethyl ether of TSG (CMTG, [HT-40] CAS 68551-04-2) and 2-hydroxypropyl ether of TSG (HPTG, [TG-30] CAS 68647-15-4) were all from SHIKIBO LTD. 3-2-6, Bingomachi, Chuo-ku, Osaka 541-8516 Japan.; poly(vinyl pyrrolidone (PVP, Kollidon 30, Mw 44–54 kDa) was from BASF, Germany; Stearic acid (SA), Sodium bicarbonate (SB), citric acid (CA), magnesium stearate and colloidal silicon dioxide were all of Ph.Eur. quality.

9.2 Methods

9.2.1 Melt granulation

SA was molten (70°C) in a beaker and the required quantity of M was added to the molten mass. The resulted mixture was stirred well to mix using a laboratory scale high mixture granulator at speed of 400 rpm (made by technical unit in Arab International University). After cooling on room temperature, the mass was passed through a 20-mesh sieve.

9.2.2 Preparation of tablet without PVP

SA and M granules were prepared by melt granulation (9.2.1). Previously prepared mixture of CA, HPMC and SB was added and the mixture was stirred well to mix. After cooling on room temperature, the mass was passed through a 20-mesh sieve, and the resulting granules were resifted on a 100-mesh sieve to remove the fines. The granules from both the 20- and 100-mesh sieves were collected and mixed with 0.4% wt/wt

magnesium stearate and 0.4% wt/wt colloidal silicon dioxide. This lubricated blend was compressed into tablets using 16-mm flat-face round tooling on a SHAKTI rotary tablet machine. Compression force was adjusted to obtain tablets with hardness in range of 5 to 14 kg/cm^2. Tablets weighed 1003±5.2 mg, and showed an average diameter of 16±0.1mm and thickness of 3.5±0.4 mm. All effervescent floating tablet (EFT) contained 500 mg M, 20mg CA, 4 mg aerosil-200 and 4mg magnesium stearate. The amount of total excipients was fixed at 503 mg and $X_1 + X_2 + X_3 = 475$ mg.

If needed, TSG or its derivatives amounts did substitute equal amounts of HPMC and mixed with the prepared mixture of CA, HPMC and SB hence tablet weight was constant.

9.2.3 Preparation of tablets with PVP

PVP was dissolved in isopropanol. The PVP solution was add to previously prepared mixture of M, HPMC, TSG, CA and SB. The resulted mixture was stirred well to mix using a laboratory scale high mixture granulator at speed 400 rpm (made by technical unit in Arab International University). Granules were dried at 40°C for 1.5 hour with residual moisture content between 0.1 and 0.3% wt/wt. The dried granules were passed through a 20-mesh sieve, and the resulting granules were resifted on a 60-mesh sieve to remove the fines. The remained granules on the top of 60-mesh sieves were collected and mixed with 2.1% wt/wt magnesium stearate and 1% wt/wt colloidal silicon dioxide.

This lubricated blend was compressed into tablets using 16-mm flat-face round tooling on a SHAKTI rotary tablet machine. Compression force was adjusted to obtain tablets hardness in range of 5 to 10 kg/cm^2. Tablets weighed 970 ± 3.0 mg, and showed an average diameter of 16±0.1 mm and thickness of 4 ± 0.2 mm.

9.2.4 Evaluation of hardness

Hardness (H) was tested using a hardness tester (ElectroLab, Mumbai, India). The results were given as mean of 6 measurements.

9.2.5 Measurement of floating lag time

Floating lag time (FLT) was measured as the time required for the tablet to raise to the surface of a beaker containing 100 mL 0.1N HCl and float (Rosa et al.1994). The results were given as mean of 6 measurements.

9.2.6 Estimation of metformin content of tablets and dissolution media

An ultraviolet (UV) spectrophotometric (T60 U UV/VIS spectrophotometer, PG instruments Ltd, Alma Park, Woodway Lane, Wibtoft. Leics. United Kingdom LE17 5BH) method based on measurement of absorption at 233 nm in water was used for the estimation of metformin according to USP 32. The method showed very good linearity (R2 > 0.9998) in the concentration range of 0–30 µg/mL. When a standard drug solution was assayed a number of times (n = 6) the relative error (accuracy) and the relative standard deviation were found to be 0.8% and 0.47% respectively. The assayed content of drug in tablets varied between 98.11% and 99.57% (mean 99.84).

9.2.7 Dissolution study

Drug dissolution was studied using a dissolution apparatus type 2 (USP 32, ERWEKA dissolution test, Heusenstamm, Germany), 900 mL 0.1N HCl at 37±0.5°C and at 100 rpm. Sink condition was maintained for the whole experiment. 10 mL of the dissolution medium were withdrawn at regular intervals and the same volume of pre-warmed (37±0.5°C) fresh dissolution medium was replaced. The samples withdrawn were filtered through a 0.45µm membrane filter (Nunc, New Delhi, India) and the drug content in each sample was analyzed after suitable dilution. The results

were given as mean of 6 measurements. $t_{60\%}$ was estimated from dissolution results fitted according to:

Efentakis et al. (2007) for tablet without PVP Eq. (9.1):

$$\frac{M_t}{M_\infty} = k_1 \times \sqrt{t} + k_2 \times t \qquad (9.1)$$

Or Higuchi (1963) for tablet with or without PVP Eq. (9.2):

$$\frac{M_t}{M_\infty} = k_1 \times \sqrt{t} \qquad (9.2)$$

M_t/M_∞ is the percentage of drug released at time (t), k_1 represents the Higuchi rate constant diffusion mechanism and k2 represent the erosion (relaxation) mechanism.

For granules dissolution apparatus type 1 (basket) was used.

9.2.8 Swelling study

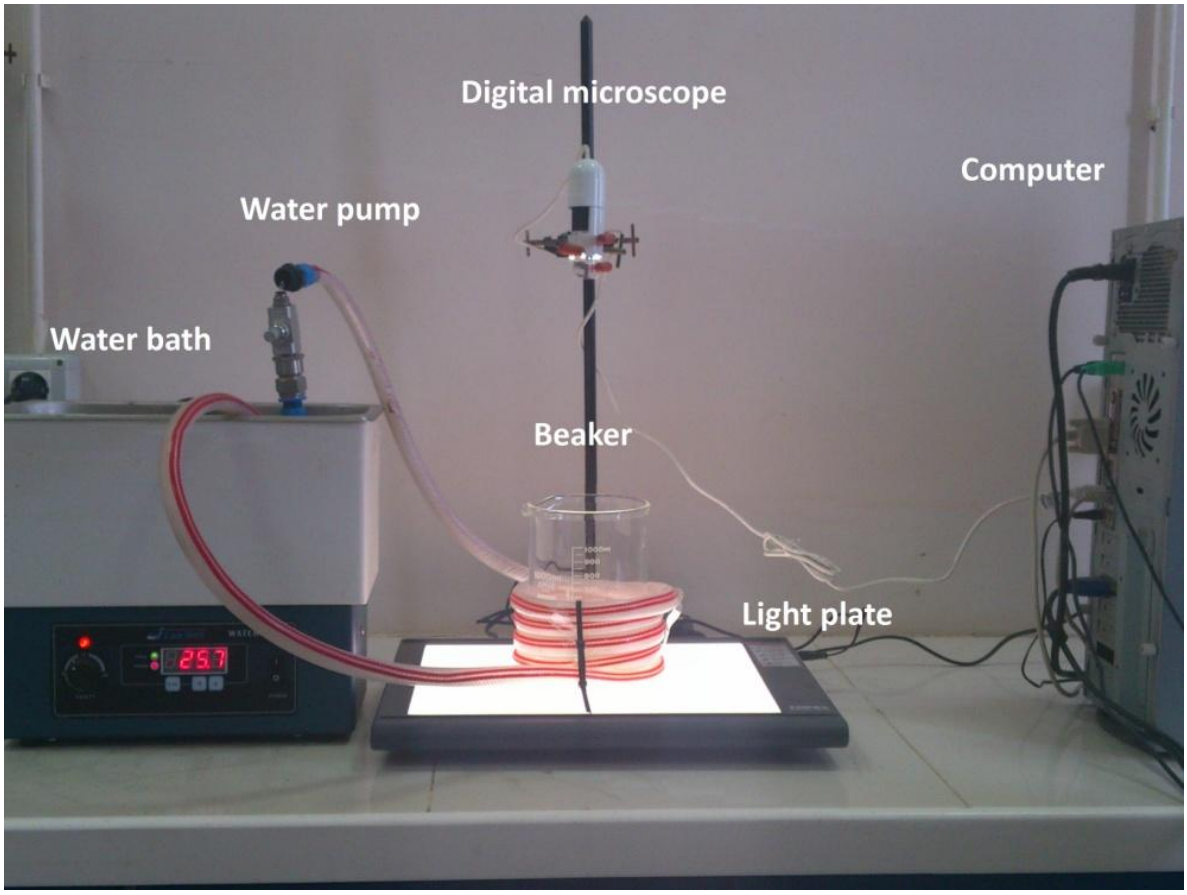

Figure 9.1. Laboratory equipment for swelling studies

For swelling studies a Digital microscope was used to take one photo each half hour for tablets during 24 hours (Figure 9.1). 4 tablets of each formula were tested where each tablet was inserted into transparent tube and brought into contact with aqueous solution of 0.1N HCl at 37°C to observe increase in thickness as described earlier by Baumgartner et al. (2002). Observations were done using a digital image analysis system (ImageJ 1.45q, Wayne Rasband, National Institutes of Health, USA) capturing images each half hour during 24 hours using Dino Digital Microscope (AnMo Electronics Corporation®). Five observations of length were carried out equally distanced along the tablet diameter (figure 9.2) to avoid mistakes caused by non homogenous swelling and the mean was further evaluated.

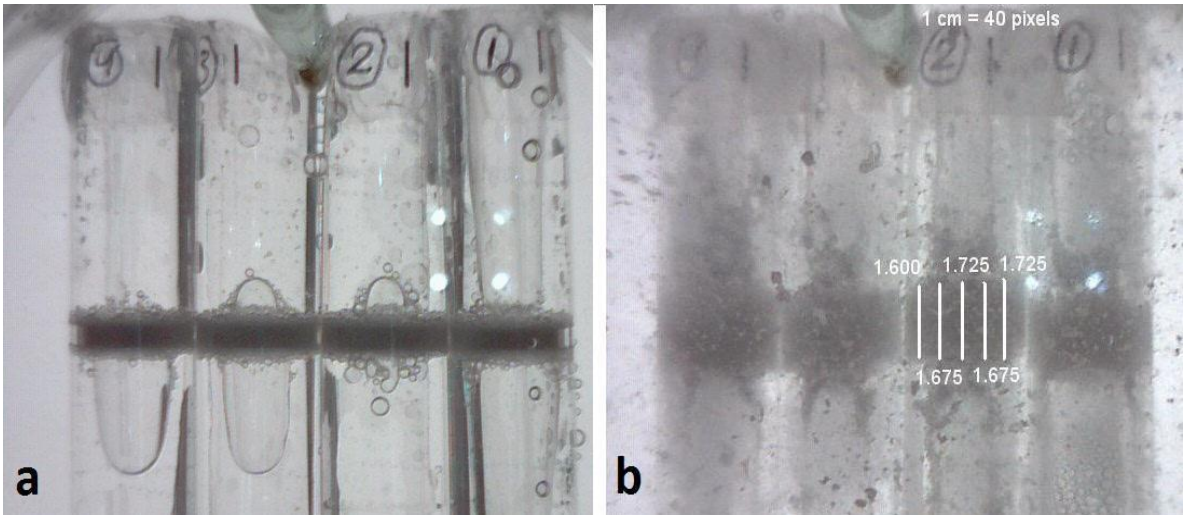

Figure 9.2. Swelling of four tablets. a) At the beginning b) After 24 hours

The dynamics of the water uptake was expressed as the weight gain (w_p) of the swelled matrix, Davidson III and Peppas (1986) calculated it as a ratio between the amount of the aqueous phase remaining in the swollen matrix for a given period of time and the initial weight of the dried matrix tablet. The kinetics of the liquid penetration into all hydrophilic matrices studied was analyzed according to Michailova et al. (2000) Eq. (9.3):

$$w_p = K_p t^{n_p} \tag{9.3}$$

Where w_p is the weight gain of the swelled matrix (g penetrant/g dry polymer); K_p, kinetic constant of aqueous solution penetrant; t, penetration time; n_p, exponent which depends on the aqueous solution penetration mechanism.

In the present work, hydrophilic polymers and other compounds formed a homogenized tablet mixture and during swelling tablet kept its cylindrical shape with constant radius (r) and increased thickness (d). Hence the penetrant volume of aqueous solution was equal to the increase of tablet volume; w_p could be calculated using Eq. (9.4):

$$w_p = \frac{w_w}{w_0} = \frac{\rho_w V_w}{\rho_0 V_0} = \frac{\rho_w (V_t - V_0)}{\rho_0 V_0} = \frac{\rho_w \pi r^2 (d_t - d_0)}{\rho_0 \pi r^2 d_0} = \frac{\rho_w}{\rho_0} \times \frac{(d_t - d_0)}{d_0} \tag{9.4}$$

Where:

w_w, ρ_w and V_w are weight, density and volume of penetrant aqueous solution of 0.1 N HCl respectively;

w_0, ρ_0, V_0 and d_0 are weight, density, cylindrical volume and thickness of initial dried tablet respectively;

V_t and d_t are cylindrical volume and thickness of tablet after t of continuous swelling respectively.

In other hand, the average percentage of tablet thickness (S_t) was the thickness of tablet after t of continuous swelling to its original thickness as described in Eq. (4):

$$S_t = \frac{d_t}{d_0} \times 100 \qquad (9.5)$$

From (2), (3) and (4) we wrote Eq. (5):

$$w_p = \frac{\rho_w}{\rho_0} \times \frac{(S_t - 100)}{100} \Rightarrow S_t = 100\frac{\rho_0}{\rho_w} \times K_p t^{n_p} + 100 \qquad (9.6)$$

Finally,

$$S_t = K_s t^{n_p} + 100 \qquad (9.7)$$

Where $K_s = 100(\rho_0/\rho_w)K_p$.

S_t was fitted over time to Eq. (6) that derived from Michailova et al. (2000) model.

9.2.9 Adhesion and mucoadhesion study

To measure the adhesive properties, a method derived from Metia and Bandyopadhyay (2008) was adapted applying shear stress horizontally and resulting in work of adhesion. The work of tablet adhesion on stainless steel (W_{ss}) and the work of tablet adhesion on gastric mucosa

(W_{gm}) were used to show the tablet adhesion properties. W_{ss} was investigated using 6 tablets from each mixture. Each Tablet was thrown in beaker that contained 300 mL of HCl 0.1 N at 37°C for 60 minutes then it was placed into beaker that contained 100 mL HCl 0.1N at 37°C on stainless steel plate under a weight 13.11g for 60 minutes. The measurement and calculation of work of shear adhesive force W_{ss} of tablet during 3 minutes were applied using the apparatus described in Figure 9.3. Same steps were applied to W_{gm} of tablet on rabbit gastric mucosa that was fixed on the stainless steel plate (Figure 9.4) The measurement device applied shear stress by moving the tablet horizontally to the load cell at a constant speed (42 μm/s) whilst adhering to stainless steel or gastric mucosa. The load cell delivered one force measure (mN) per 9.6 ms with a deviation of ± 1.96 mN. The work of adhesion per unit area (W_{ss} or W_{gm}, J/m^2) was the quotient of the area (J) under a 3 min force versus distance profile and the contact area (m^2) (Thirawong et al. 2007).

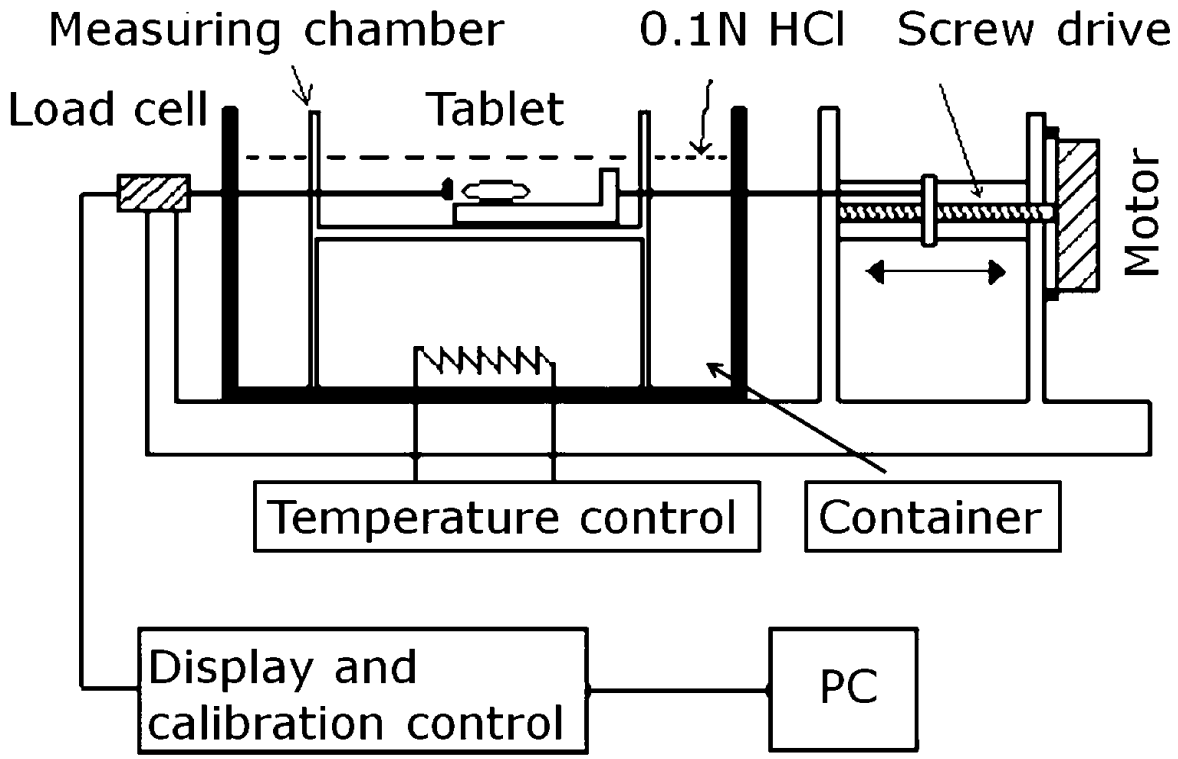

Figure 9.3. Diagram of laboratory equipment for adhesion measurement on stainless steel.

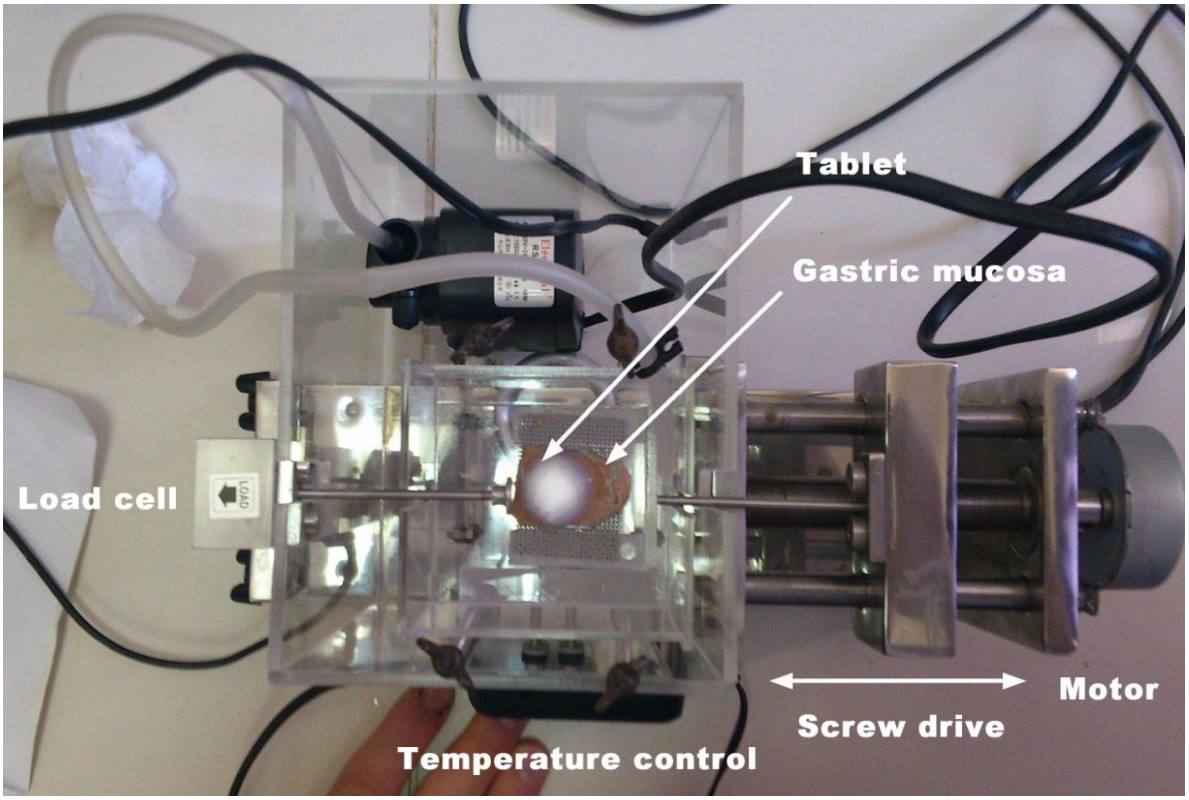

Figure 9.4. Lab equipment for adhesion measurement on rabbit gastric mucosa.

9.3 Experimental designs and statistics

9.3.1 Experimental design for optimizing the effervescent tablet

To optimize the effervescent tablet for its H, FLT and $t_{60\%}$ proprieties to have their values in 10.2 – 20.3 Kg/cm^2, 0 - 120 seconds and 3 - 4.5 hours respectively. Fraction of amounts of SA, HPMC and SB were used as factors in a mixture design. This was a randomized run of simplex lattice design (Rajab et al. 2010) with three-component mixture for which the number of equally spaced levels for each component is four. In addition the design was augmented by adding a middle point between the center and each edge to raise the precision and predictability of the design. The coded values SA + HPMC + SB = 1 and the actual values SA (mg) + HPMC (mg) + SB (mb) = 475 mg.

The experimental result of H, FLT and $t_{60\%}$ were fitted to cubic model because it has the lowest predicted residual sums of squares (PRESS)

comparing to linear, quadratic and special cubic. The cubic model equations were used simultaneously to reach the optimum values for H, FLT and $t_{60\%}$ into their specified ranges and to determine the best ranges for SA, HPMC and SB.

9.3.2 Experimental design to evaluate the combinatorial effect of PVP, TSG and HPMC

To evaluate the combinatorial effects of the polymers PVP, TSG and HPMC a mixture design of experiment was used. This was the same as explained in 9.3.1 but without the need to be augmented. The dependent variables were H, FLT, $t_{60\%}$, S, W_{ss} and W_{gm}. Where in coded values PVP + TSG + HPMC = 1 and in actual values PVP (mg) + TSG (mg) + HPMC (mg) = 300 (mg).

To assure that results of simplex lattice experimental design had significant variation, unequal variances t-test of difference (two tails, α = 0.05) was applied to all possible pairs of means in each in-vitro performance group. To show the combinatorial effects of the polymers and using coded level of PVP, TSG and HPMC as parameters, experimental results of H, FLT and W_{ss} were first fitted to linear, quadratic, special cubic and cubic models . The appropriateness of models, were evaluated using p-Value of sequential model sum-of-squares analysis to limit the number of interaction terms in model, coefficient of multiple determination (R^2, α = 0.05) as an indicator to goodness of fit, adjusted R^2 to eliminate the effect of different number of parameters that could influence on R^2, predicted R^2 to indicate the capability of the regression model to predict new observations. To refine the selected models, t-test of slopes of regression equation was used to keep only the significant terms (α = 0.05). The reliability of the models was tested using standardized main effects (SME) (Nutan et al. 2005) that was calculated by dividing the main effects with the standard error of the main effects, R^2 coefficient of multiple determination (R^2, α = 0.05), p-Value of lack of fit (α = 0.05). The mathematical models were fitted using linear least square method

implemented in Microsoft Excel (2007) and Design Expert® (trial version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN). Prior to data analyze the normal distribution of the data was confirmed using the Anderson–Darling normality test at significance level α = 0.05.

To discriminate between the hydrophilic and more hydrophobic tablets the amount of TSG in the tablets was kept accordant as much as possible for the pairs compared, pair comparison was performed on three levels (1.7%, 3.4% and 5.1%) applying the t-test of differences (two tails, α = 0.05).

9.3.3 Spearman´s rank correlation

To compare the adhesive properties depending on substrate, Spearman's rank correlation coefficient was calculated (two tails, α = 0.05) between W_{gm} and W_{ss} and a simple regression line between the two variables was fitted. Design Expert® (trial version 8.0.7.1) was used to generate response surfaces to visualize the effect of factors on responses.

9.3.4 Protocol of biostudy and statistical methods

The assessment of the products included in this study was designed so that an open label, single dose of the test product Diaphage® GR 500 mg tablet was compared with the reference product Glucophage® XR 750 mg in 6 healthy male subjects. Subjects were hospitalized the night before the start of the study and they received a standard supper before 7.30 p.m. and they fasted overnight for ten hours, and given a single oral dose of either test or reference drug in accordance with a randomization code. this was performed by previously labeled and packed of either the test or the reference. A blood sample was withdrawn through a cannula placed into a suitable forearm or hand vein, before subjects having drug samples, then after post –dosing blood samples were drawn at the following time intervals 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 8.00, 10.00 and 12.00 hours post drug administration.

A standard breakfast and a standard lunch were offered to all subjects 4 and 9 hours respectively after drug intake. The subjects were supervised throughout the period of the study.

The blood samples (5 ml) were collected into tubes using heparin as anticoagulating agent. After sampling, the tubes with blood were immediately centrifuged (4000 rpm, room temperature, for 5.0 min), the separated plasma were transferred into a polypropylene tubes, immediately frozen and stored at the clinic at a temperature below –20°C. The plasma samples were transferred frozen from the hospital to the laboratories of Jordan Center for Pharmaceutical Research and kept frozen at a minimum of –20°C till analyzed.

The determination of metformin plasma concentrations was performed by means of a validated HPLC assay method, at Jordan Center for Pharmaceutical Research.

Only subjects presenting all of the following inclusion criteria were enrolled in the present trial:

• Males, age between 18 and 50 years.

• Physically and mentally healthy as judged by means of a medical and standard laboratory examination.

• Weight not less than 60 kg

• Body weight within = ±10% of the ideal body weight in relation to height according to the Body Mass Index : {BMI = Weight (kg) / Height (m) 2 ,(17.1 to 28.6) }

• Informed consent given in written form according to section 17.3 of the study protocol.

Subjects presenting any of the following exclusion criteria were not included in the trial:

• Allergic diathesis or any clinically significant allergic disease.

• History of allergic response to metformin, or any of excipients.

• The subject is a pregnant female (positive urine/blood pregnancy test) or lactating female.

• Drinking alcohol or use street drugs.

• Having any heart problems and/ or low or high blood pressure or have had a stroke.
• History of seizures.
• Having diabetes.
• History of low white blood cell count which may or may not have been caused by other medicines.
• Exercise vigorously or work in hot or sunny places.
• Presence or history of depression or have other mental illnesses.
• Presence or history of any kidney disease or kidney problems.
• Presence or history of any liver disease or liver problems.
• Presence or a history of clinically significant cardiovascular, renal, hepatic, pulmonary, metabolic, endocrine, hematological, gastrointestinal, neurological, psychiatric or other major disease.
• Clinically relevant abnormalities at physical examination, ECG, or laboratory tests.
• Any chronic disease which might interfere with absorption, distribution, metabolism or excretion of the drug.
• Clinically significant illness within 4 weeks before the start of the study.
• Laboratory values outside normal range with clinical relevance at entry examination.
• Intake or administration of any prescribed systemic or topical medication within 2 weeks prior to the start of the study.
• Concomitant intake or administration of any systemic or topical drugs.
• Treatment with any investigational drug (i.e. drug not yet approved) in the last 3 months before beginning of the trial.
• Participation in another clinical trial within the last 2 months.
• Treatment with drugs known to alter the major metabolic systems such as barbiturates, phenothiazines, cimetidine, omeprazole etc. within the last 30 days.
• Major surgery of the gastrointestinal tract except for appendectomy.
• Donation of blood or plasma within the last two months.

• Supine blood pressure, after resting for 5 min, higher than 140 /90 or lower than 90/60 mmHg.
• Supine pulse, after resting of 5 min, outside the range of 60-90 beats /min.
• Body temperature higher than 37.7°C or lower than 36.4°C.
• Smoking of more than 10 cigarettes or equivalent per day.
• Consumption of more than 4 cups of Coffee or equivalent /day or 24 hours before dosing and through the hospitalization periods.
• Vegetarian.
• Knowledge to have a hepatitis B, C and HIV infection or carrier of the respective antigens (HBs Ag, positive).
• Evidence of an uncooperative attitude.
• Alcohol Abuse i.e. consumption of more than 10 units of alcohol per week or a history of alcohol of alcoholism or drug/chemical abuse.
• Legal incapacity and /or other circumstances rendering the Subject unable to understand the nature, scope and possible consequences of the study.
• Vigorous exercise was undertaken beginning 2 days before the initial screening laboratory test until after the final laboratory safety tests.

Demographic characteristics were as following: 6 healthy male subjects aged 19 - 36 years (mean 27.75 ± 6.04), weighed 60-80 kg (73.13 ± 7.53), height 170 - 180 cm (mean 174 ± 4) and body mass index (BMI), 18.52 – 27.68 (mean 24.21 ± 2.90), were enrolled in the study.

For each subject and each treatment, the following pharmacokinetic parameters were calculated by Noncompartmental analysis using the software Kinetica 2000 version 4.2, Innaphase Corporation, France (Appendix 26).

AUC_{0-t} = AUC_{last}, Area under the concentration - time curve, calculated by the trapezoidal rule (time 0.00 h to last measurable concentration, C_t).

$AUC_{0-\infty}$ **=** AUC_{tot}, The area under the plasma concentration versus time curve from time (0) to infinity. $AUC_{0-\infty}$ was calculated as the sum of the

AUC_{0-t} plus the ratio of the last measurable plasma concentration to the elimination rate constant.

$AUC_{0-\infty} = AUC_{0-t} + C_t/K_{el}$

C_{max} **=** Observed maximal concentration after administration**.**

T_{max} **=** Observed time of maximal concentration. If the maximum value occurs at more than one time point, T_{max} is defined as the first time point with this value.

$T_{1/2}$ = Terminal half -life calculated according to $In(2/ K_{el})$**.**

K_{el} **=** Apparent first-order elimination or terminal rate constant calculated from a semi-log plot of the plasma concentration versus time curve. The parameter was calculated by linear least-squares regression analysis using the last three (or more) non-zero plasma concentrations. ANOVA 2 way test at significance level $\alpha=0.05$ between Diaphage® GR 500 mg and Glucophage® was conducted on the natural logarithm of C_{max} and AUC_{0-t}.

10 Summary

10.1 English Version

Metformin, alone or in combination with other oral anti-diabetic agents, has become the standard of care for the first-line treatment of type 2 diabetes patients.

There are numerous solid dosage forms containing metformin currently available in the market. Besides the immediate releasing (IR) tablets four types of extended release (XR) tablets are sold. All of them seek to lower the blood glucose but none of them consider the fact that misalignment of internal circadian oscillators with the external environment leads to deleterious health consequences and has long been associated with increased morbidity and mortality in humans (Reddy and O'Neill 2010).

In addition, the fluctuation of blood glucose is a significant independent risk factor of mortality especial in critically ill patients (Tang and Gu 2012). In addition, some investigations proposed that metformin could modulate the circadian system.

Taken together, the apparent beneficial association between targeted modulation of the circadian system and the whole-body metabolic state suggests that pulsatile metformin administration could be a promising approach for the treatment of obesity and type 2 diabetes (Viollet et al. 2012).

The general objective of this work was to develop a tablet of metformin that realizes drug release profile that follow the circadian variation of glucose levels (working hypothesis).

Struggling to realize this hypothesis a gastric retentive tablet that uses the effervescent floating principle was selected and combined with

bioadhesive properties of the tablet swollen within the gastro-intestinal tract.

Materials that occurring more or less in nature like stearic acid, tamarind seed gum were blended in different fractions with semi synthetic ones as hydroxypropyl methylcellulose and Poly(vinyl pyrrolidone) to prepare batches of tablets.

The melt granulation and non-aqueous granulation processes were used to protect metformin from humidity. New apparatus to measure adhesion was built even new standards were proposed to realize some of in vitro performances measurements like swelling and adhesion. In this work, a statistic experimental design called simplex lattice for mixture design was used in part, complete or extend to compare the effects of different elements.

These investigations confirmed the working hypothesis, improving a known gastric retentive tablet principle, the effervescent floating tablet, by bioadhesive components. The introduction of stearic acid using melting process led to enhanced physical properties of the tablets.

Also, the optimization of the manufacturing process revealed the possibility to use stearic acid as release controller of metformin from the tablets.

Furthermore, the use of a fraction of tamarind seed gum or its derivatives instead of hydroxypropyl methylcellulose in the tablets was seen helpful to adjust swelling and metformin release whilst adhesion of the swollen tablets remains unchanged.

This finding resulted in deeper insight of the combinatorial effects of tamarind seed gum and hydroxypropyl methylcellulose as more soluble polymers. Poly(vinyl pyrrolidone) as component of the tablets influenced the in vitro performance significantly. As result, the interaction between hydroxypropyl methylcellulose and Poly(vinyl pyrrolidone), previously

documented by Chan et al. (2003), was verified for this tablet composition beside a possibility of further interactions.

The new apparatus for adhesion measurement applied the shear force horizontally. The results of these measurements have demonstrated a moderated correlation between adhesion on stainless steel and mucoadhesion on rabbit gastric mucosa. The results of the investigations of in vitro performance of the tablets were partially confirmed by a pilot biostudy on human subjects showing bioavailability for the final tablet resulting from this work (Diaphage®GR 500 mg) better than the reference drug Glucophage®XR 750 mg.

A two-pulse releasing tablet for once daily use of anti-diabetic drugs like metformin resulted. The tablet provides two peaks in the blood plasma concentration versus time profiles in humans. This profile is mimicking the desirable biologic rhythm. This approach is inventive (patent announced: Rajab M et al. 2012c). The new formulation developed here may increase the therapeutic efficacy of metformin and improve the patient compliance due to once daily administration of a lower dose of the drug. This may reduce side effects of metformin.

10.2 German version (Zusammenfassung)

Metformin ist allein oder in Kombination mit anderen oralen Antidiabetika zum „Goldstandard" der Therapie des Diabetes Typ 2 geworden.

Zahlreiche feste Arzneiformen mit Metformin sind derzeit im Markt verfügbar. Neben schnell freisetzenden (IR) Tabletten werden vier Arten von Tabletten mit verzögerter Freisetzung (XR) verkauft.

Alle genannten Arzneiformen streben eine Senkung des Blutzuckers an aber keine berücksichtigt die Tatsache, dass eine falsche Ausrichtung des internen zirkadianen Schwingkreises gegenüber der externen Umwelt schädliche gesundheitliche Konsequenzen hat und auf Dauer mit ansteigender Krankhaftigkeit und Sterblichkeit der Menschen verbunden ist (Reddy und O'Neill 2010).

Außerdem ist die Schwankung des Blutzuckers ein unabhängiger signifikanter Risikofaktor der Sterblichkeit besonders für schwerkranke Patienten (Tang und Gu 2012). Weiterhin haben einige Untersuchungen gezeigt, dass Metformin das zirkadiane System modulieren könnte.

Alles zusammen genommen deutet sich an, dass es eine scheinbar günstige Verbindung zwischen zielgerichteter Modulation des zirkadianen Systems und des metabolischen Zustands des gesamten Körpers gibt, so dass eine rhythmische Gabe von Metformin ein vielversprechender Ansatz für die Behandlung der Fettleibigkeit und des Typ 2 Diabetes sein könnte (Viollet et al. 2012).

Das allgemeine Ziel dieser Arbeit bestand in der Entwicklung einer Metformin-Tablette, die ein Arzneistoff-Freisetzungsprofil realisiert, das der zirkadianen Schwankung des Glukose- Blutspiegels folgt (Arbeitshypothese).

Im Bemühen diese Hypothese zu realisieren wurde eine schwimmfähige gastro-retentive Brausetablette ausgewählt und mit bioadhäsiven Eigenschaften der im Magen-Darmtrakt gequollenen Tablette kombiniert.

Materialien die mehr oder weniger in der Natur vorkommen, wie Stearinsäure, Gummi aus Tamarindensamen, wurden zur Herstellung der verschiedenen Tablettenchargen in verschiedenen Verhältnissen mit halbsynthetischen Materialien wie Hydroxypropylmethylzellulose und Polyvinylpyrrolidon gemischt.

Um Metformin vor Feuchtigkeit zu bewahren wurden die Schmelzgranulation und die wasserfreie Granulation benutzt. Zur Messung der Haftung wurde ein neuer Apparat gebaut und zur Untersuchung der in vitro Effizienz, wie Quellung und Haftung, wurden neue Standards aufgestellt. Ein statistisches Experimentaldesign, das sogenannte Einfach-Gitter-Mischungsdesign, wurde ganz oder teilweise benutzt, um die Auswirkungen der verschiedenen Elemente zu untersuchen.

Die Untersuchungen bestätigten die Arbeitshypothese, dass das bekannte Prinzip der gastro-retentiven Tablette, der schwimmfähigen Brausetablette, durch bioadhäsive Komponenten verbessert werden kann.

Die Einführung von Stearinsäure führte zusammen mit dem Schmelzprozess zu verbesserten physikalischen Eigenschaften der Tabletten.

Ebenso brachte eine Optimierung des Herstellungsprozesses die Möglichkeit zu Tage, Stearinsäure zur Kontrolle der Metforminfreigabe aus den Tabletten zu nutzen.

Weiterhin war die Benutzung eines Anteiles Gummi aus Tamarindsamen oder Derivaten davon anstelle der Hydroxypropylmethylcellulose in den Tabletten hilfreich um die Quellung und Metforminfreigabe einzustellen während die Adhäsion der gequollenen Tabletten unverändert blieb.

Dieses Ergebnis ergab tiefere Einsicht in die kombinatorischen Auswirkungen von Gummi aus Tamarindsamen und Hydroxypropylmethylcellulose als den eher löslichen Polymeren. Poly(vinyl pyrrolidone) als Bestandteil der Tabletten beeinflusste signifikant die in vitro Güte. Als Ergebnis wurden Wechselwirkung zwischen Hydroxypropylmethylcellulose und Poly(vinyl pyrrolidone), die vorher durch Chan et al. (2003) dokumentiert worden waren, für diese Tabletten-Zusammensetzung ungeachtet der Möglichkeit weiterer Wechselwirkungen verifiziert.

Der neue Apparat für die Messung der Haftkraft wendet die Scherkraft horizontal an. Das Ergebnis dieser Messungen hat eine moderate Korrelation zwischen der Haftung auf rostfreiem Stahl und der Schleimhauthaftung auf der Magenschleimhaut von Kaninchen ergeben.

Die Ergebnisse der in vitro Güte der Tabletten wurden teilweise bestätigt durch eine Pilot-Biostudie an menschlichen Versuchspersonen, die zeigte, dass die aus den Untersuchungen dieser Arbeit letztendlich resultierende Tablette (Diaphage®GR 500 mg) eine bessere Bioverfügbarkeit aufweist als das Vergleichspräparat Glucophage®XR 750 mg.

Heraus gekommen ist eine rhythmisch freisetzende Tablette für den einmal täglichen Gebrauch eines antidiabetischen Medikaments wie Metformin. Die Tablette verspricht für die Anwendung am Menschen zwei Gipfel in der Darstellung der Blutplasmakonzentration gegen die Zeit.

Dieser Verlauf ahmt den gewünschten biologischen Rhythmus nach. Diese Herangehensweise ist erfindungsgemäß (Patent angemeldet: Rajab M et al. 2012c). Die hier entwickelte neue Formulierung könnte die therapeutische Wirksamkeit von Metformin verbessern und durch die einmal tägliche Anwendung einer geringeren Dosierung des Arzneimittels die Patienten Compliance ansteigen lassen. Das kann die Nebenwirkungen von Metformin herabsetzen.

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List of own publications

- Patent and Invention :

Rajab M, Dittgen M, Jouma M, Neubert RHH, Maabared AG, AIKamal MH (2012) Two-pulse releasing pharmaceutical compositions for once daily use of anti-diabetic drugs like metformin. German patent and trademark office. Application no. 102012102414-6.

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Declaration of interest

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However, the candidate himself and alone is responsible for the content and for writing this paper.