Bull. Pharm. Sci., Assiut University, Vol. 30, Part 2, 2007, pp. 193-212.

THE EFFECT OF BINDER TYPE AND MAJOR DILUENT ON THE MIGRATION AND BIOAVAILABILITY OF RIBOFLAVIN SODIUM PHOSPHATE DURING TABLET MAKING

Hassan M. ELSabbagh, Ahmed T. Nouh, Osama A. Soliman and Mariza F. Boughdady

Department of Pharmaceutics, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt

إن هجرة الأدوية القابلة للذوبان وذات الجرعة المنخفضة او المواد الملونة أثناء تجفيف حبيباتها الرطبة، قد تؤدى إلى حركة المادة الفعالة الذائبة من حبيبة إلى أخرى مما يتسبب في سوء توزيع المادة الفعالة أو المادة الملونة في الحبيبات ومن ثم في الأقراص المصنعة منها. ونتيجة لذلك، قد يحدث إختلاف كبير في جرعة الدواء من قرص إلى آخر حتى في نفس تشغيلة الأقراص الواحدة وخاصة في حالة الأدوية ذات الجرعة الصغيرة وذات الفاعلية العالية. أما هجرة اللون فقد تتسبب في الحصول على أقراص غير مقبولة الشكل. وفى هذه الدراسة، تم تحضير حبيبات مختلفة تحتوي على ريبوفلافين فوسفات الصوديوم باستعمال صواغات مختلفة (لاكتوز أحادى الماء وفوسفات الكالسيوم ثنائي القاعدة)، ومحاليل مائية لروابط متنوعة وهي عديد فينيل البيروليدون ك٢٥ (٥ و١٠ و١٥%) والميثيل سيليولوز (٠,٥ و١ و١,٥%) والهيدروكسى بروبيل ميثيل سيليولوز (٠,٥ و١ و١,٥%) والجيلاتين (٢,٥ و٥ و١٠%) . ثم بعد ذلك تم تجفيف الحبيبات الرطبة عند درجتي حرارة ٥٠°م و٧٠°م، ثم حساب قيمة معامل الهجرة للدواء فى الحبيبات الجافة. وبعد ذلك تم كبس هذه الحبيبات الجافة فى صورة أقراص وتقييمها فيزيائياً من حيث المظهر العام، محتوى الدواء، الوزن، الصلابة، الهشاشة، التفتت ومعدل الانطلاق المعملي للدواء. وفى النهاية تم أيضاً اختيار بعض الأقراص ذات الخواص الفيزيائية المتباينة لتقييمها حيوياً للوقوف على أفضلها من ناحية الإتاحة الحيوية للعقار. أوضحت النتائج أن هجرة الدواء كانت أقل في حالة الحبيبات المحضرة باستخدام فوسفات الكالسيوم ثنائي القاعدة عن تلك المحضرة باستعمال اللاكتوز وأن زيادة لزوجة الرابط وزيادة تركيزه تؤدى إلى تقليل هجرة الدواء. كما أن هجرة الدواء حدثت بدرجة أقل عند تجفيف الحبيبات عند درجة حرارة ٧٠°م. ولقد تبين أيضاً الحصول على حبيبات متجانسة من حيث توزيع الدواء ولونه باستعمال الجيلاتين بنسبة ١٠% كرابط أياً كان نوع الصواغ المستخدم حيث أظهر هذا الرابط أقل قيمة لمعامل هجرة الدواء. وقد أوضحت نتائج الدراسة أيضا أن درجة عدم تجانس اللون في القرص تتناسب عكسياً مع تركيز الرابط. حيث أظهرت الأقراص

Received in 18/10/2007, Received in revised form in 26/12/2007 & Accepted in 27/12/2007

المحضرة باستعمال ٠,٥% ميثيل سيليولوز اعلى نسبة هشاشة (٢,٩) ، اقل درجة صلابة (٤,٥) وأقل زمن للتفتت (١,٦٨ دقيقة). كما كانت الأقراص تفتقر إلى التوزيع المتجانس للدواء. بينما الأقراص المحضرة باستعمال ١٠% جيلاتين أظهرت توزيعاً متجانساً لدواء وأقل معدل إذابة وأقل نسبة هشاشة (٠,٨٧١)، أعلى درجة صلابة (٧,٩٥) وأعلى زمن للتفتت (٢٥ دقيقة) مقارنة بالأقراص الأخرى. وبإختيار الأقراص المحضرة باستعمال ١٠% جيلاتين وتلك المحضرة باستعمال ٠,٥% ميثيل سيليولوز لدراسة الإتاحة الحيوية على ستة متطوعين ذكور أصحاء، تم تعيين إفراز الريبوفلافين في البول. وقد أثبتت النتائج أن الأقراص المحضرة باستعمال ١٠% جيلاتين كان لها أقل معدل إفراز للدواء في البول وأعلى قيمة للزمن اللازم لأعلى تركيز (١,٥ ساعة). أما الأقراص المحضرة باستعمال ٠,٥% ميثيل سيليولوز كان لها أعلى معدل إفراز للدواء وأقل زمن لازم لأعلى تركيز (٠,٥ ساعة). كما وجد أيضاً ارتباط جيد بين الإتاحة المعملية للدواء والإتاحة الحيوية له.

The migration of riboflavin sodium phosphate (RSP) upon drying of its wet granules was studied through the formulation of granules using different diluents (lactose monohydrate and anhydrous dibasic calcium phosphate), different binders of different concentrations; polyvinyl pyrrolidone (PVP k_{25}), methylcellulose (MC), hydroxypropylmethyl cellulose (HPMC) and gelatin at different drying temperatures (50°C and 70°C). The prepared granules were compressed into tablets and evaluated. In vitro drug release from the formulated tablets was performed. In addition, in vivo study was conducted on some selected tablet batches. The results showed that, the granules prepared with dibasic calcium phosphate showed lower migration for the drug than those prepared with lactose. Also, drug migration decreased with increasing the binder concentration and viscosity. The degree of tablet mottling was inversely proportional to the binder concentration. Tablets prepared with 10% w/w gelatin were found to be the least mottled ones. In addition, they showed the least friability percentage, the highest hardness value and the highest disintegration time. Tablets prepared with 0.5% MC showed the highest dissolution rate, however, those prepared with 10% gelatin had the lowest dissolution rate. Generally, increasing the binder concentration resulted in slowing the in vitro drug release from tablets. The in vivo study showed that, tablets prepared with 10% w/w gelatin showed the lowest excretion rate and the highest T_{max} (1.5 hours). Meanwhile, tablets prepared using 0.5% w/w MC exhibited higher excretion rate and T_{max} of 0.5 hour.

INTRODUCTION

Compressed tablets are one of the most widely used dosage forms for the administration of orally effective therapeutic agents. They must be uniform in weight and in drug content of the individual tablet. Also, they must be elegant in appearance and must have the characteristic shape, color, necessary to identify the product. Owing to their wide-ranging physicochemical and mechanical properties, pharmaceutical powders and their blends frequently exhibit poor flow and compaction behavior[1]. Thus, an intermediate step, granulation is usually required in solid dose manufacture to produce a free-flowing material with good compression characteristics. Wet granulation has been, and continues to be, the most widely used agglomeration process[2]. But migration of soluble, low-dosage, highly potent drugs or of colored additives upon drying of the wet granules may represent a serious problem. This problem is the movement of the solutes from granule to granule, upon drying, which may result in gross maldistribution of the active drug or the colored additive. As a result, excessive dose variation may occur within the same batch of tablets. Also, the migration of the color may give rise to a tablet with a mottled appearance.

The main target of this work, is to prepare riboflavin sodium phosphate granules, using various diluents, binders, and drying temperatures. Studying the effect of these variables on the extent of riboflavin sodium phosphate migration upon drying of its wet granules is also of our interest. In addition, the dried granules will be prepared in tablets form, in order to study the effect of drug migration on the physical properties of these tablets. *In vivo* evaluation of the prepared tablets will also, be investigated.

EXPERIMENTAL

Materials

Riboflavin sodium phosphate (Certa, Belgium). Lactose mono-hydrate, gelatin, H_2O_2 (30%), and absolute ethanol (Adwic, EL-Nasr Pharmaceutical chemicals Co., Cairo, Egypt). Calcium phosphate anhydrous and Talc (El Gomhouria Co. Egypt). Polyvinyl pyrrolidone (PVP k25), methyl cellulose, and hydroxypropyl methyl cellulose (Memphis Pharm. Co. Egypt). Benzyl alcohol (Rayasan Chemicals, India).

Equipment

USP standard sieves, Hot air ovens (Heraeus GS model B 5042, Gering model SPA-Gelman, Instrument No. 16414, Germany). U.V. spectrophotometer (Jasco, V-530, Japan). Rotary viscometer (Haake Inc., Germany). MSE Minor Centrifuge (MSE scientific instruments, Manor Royal, Crawley RH/0200 sussex, England). Single punch tablet machine, tablet hardness tester, Roche friabilator, tablet dissolution test apparatus USP I, and USP disintegration apparatus (Erweka-Apparatebau, G.m.b.H., Germany). Micrometer (Mitutoyo

Corporation, Japan). thermostatically controlled shaking water bath (Grant instrument Cambridge Ltd., Barrington Cambridge (B2, 5002, England). Luminescence spectrofluorometer (Perkin-Elmer model LS 45, Germany).

Methodology

Effect of binder type and major diluent on riboflavin sodium phosphate migration during drying of tablet granules

Preparation of different binder solutions and determination of their viscosity

Four types of aqueous binder solutions in different concentrations were prepared. These binders are; PVP k_{25} (5, 10, and 15% w/w), MC (0.5, 1, and 1.5% w/w), HPMC (0.5, 1, and 1.5% w/w) and gelatin (2.5, 5, and 10% w/w).

The viscosity values of the prepared binder solutions were determined using rotary viscometer which was thermostatically controlled at 30°C ± 0.5°C.

Preparation of riboflavin sodium phosphate wet granules

Migration of RSP was studied through the preparation of RSP-containing granules using different diluents, binders and drying temperatures. Two formulations were prepared; formula L and formula C. Their constituents are illustrated in Table (1)

Table 1: Composition of various formulations of granules containing riboflavin sodium phosphate.

Formula	Ingredient	Quantity per 500 mg of granules (weight of one tablet)
(L)	RSP	2 mg
	Lactose monohydrate	483 mg
	Binder solution	q.s.
(C)	RSP	2 mg
	Anhydrous calcium phosphate	483 mg
	Binder solution	q.s.

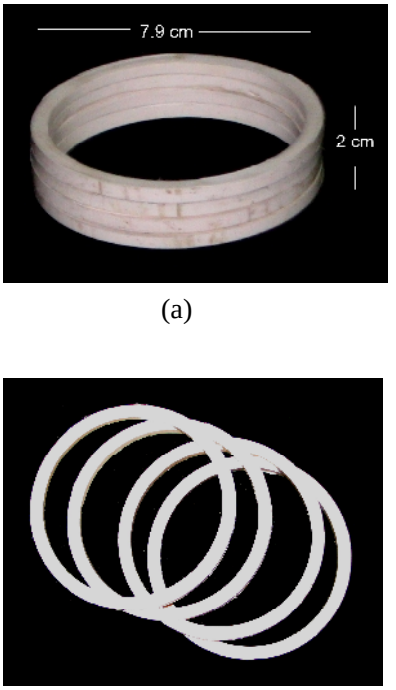
For each batch, the calculated amount of the drug was mixed geometrically with the major diluent (lactose monohydrate, in case of formula L), or (anhydrous calcium phosphate, in case of formula C). Sufficient quantity of the prepared binder solution was then used for wet massing of the mixed powders. The wet mass was kneaded for 10 min. until homogenous colored material was obtained. Wet screening was accomplished by passing the wet granulated mass through a 2 mm sieve (mesh number 10). A control experiment was performed for both lactose and calcium phosphate by applying the same procedure but with the granulating fluid being water only.

Drying of riboflavin sodium phosphate wet granules

For each batch, the wet granules were divided into two equal portions and placed in two similar drying cells. One cell was dried at 50°C and the other was dried at 70°C in two drying ovens for 24 hours.

Drying cell

Riboflavin sodium phosphate wet granules were dried in a special drying cell consisting of four concentric rings to facilitate the determination of RSP concentrations at various depths through the granulation bed. The height of each layer is 0.5 cm with a total height of 2 cm. The cell is open on both sides of the cylinder formed by the layers; the opening was 7.9 cm in diameter (Figure 1).



(a)

(b)

Fig. 1: The drying cell employed for drying of wet granules.
a- Assembled drying cell.
b- Partially disassembled drying cell.

When used for migration studies, the drying cell was placed on a drying oven tray lined with a paper. The granulation in the bottom layer was exposed to the paper during the drying process. The wetted granules, after being wet sieved, was filled into the drying cell with a spatula.

Determination of RSP concentration in different layers of the granules

Sampling procedure

After drying, the top ring of the drying cell was carefully removed. Then, the top layer of the dried bed was removed by a flat blade, mixed thoroughly, and sampled for determination of drug concentration. Three portions, weighing 100 mg each, were accurately weighed from the top layer of the dried bed and used for subsequent drug concentration determination. The procedure was repeated for the core layers (second, and third layers), and for the bottom layer also.

RSP assay method

i) For lactose granules (formula L)

Each sample was dissolved in 25 ml of distilled water. The solutions were protected from light to avoid drug degradation. The absorbances of the dissolved samples were measured at the predetermined λ_{max} (266 nm), using distilled water as a blank. The quantity of RSP, in each layer, was determined and the percentage of drug in each layer was calculated.

ii) For calcium phosphate granules (formula C)

Each sample was shaken with 25 ml of distilled water. The mixtures were then filtered through 0.45 µm millipore filter, and the concentration of RSP in the filtered samples was measured at 266 nm using distilled water as a blank. The quantity of RSP, in each layer, was determined and the percentage of drug in each layer was calculated.

The same procedure was used for determining the drug percentages in the different layers for the control experiments.

Data treatment

To specify the extent to which the drug migrated with a numerical value, the concept of a coefficient of migration was employed. It was developed by Warren and Price, (1977 a)[3]. Since there are four layers in the drying cell, there can be six layer comparison classifications, *i.e.*, a comparative difference, sign ignored, of the average assay values of layer 1 to layer 2, layer 1 to layer 3, layer 1 to layer 4, layer 2 to layer 3, layer 2 to layer 4, and layer 3 to layer 4. These layer differences were determined relative to the amount of drug recovered and potentially present in any two layers. In general, then, the equation for calculating the comparative difference (D) between two layers (j and j´) is:

$$D_{j-j'} = \frac{\left|L_j - L_{j'}\right|}{\frac{2\sum_{j-1}^{N} L_j}{N}}$$

Where; L_j represents the average of three assay values in a given layer.

$L_{j'}$ is the average assay value in another layer.

$\Sigma^{N}_{j=1}\ L_j$ is the sum of the average assay values for *N* layers.

N is the number of layers in the drying cell.

It is postulated that, the drug percent of the most divergent system using an open drying cell after drying can be represented as 200%, 0%, 0%, and 200% for layers: 1 (upper layer), 2 (second layer), 3 (third layer), and 4 (base layer), respectively, where the drug has migrated from the two intermediate inner layers (second and third layers) to the two exposed evaporating surfaces (upper and base layers). Using this equation, the classification differences would be: D_{1-2} = (200-0)/200 =1, D_{1-3} = (200-0) /200 =1, D_{1-4} = (200-200)/200 = 0, D_{2-3} = (0-0)/200 =0, D_{2-4} = (0-200) /200 =1, D_{3-4} = (0-200)/200 =1, and total = 4. So, the coefficient of migration is 4/4 = 1.

On the other hand, the most uniform system in a migration study using an open drying cell after drying can be represented as layer 1 = layer 2 = layer 3 = layer 4 = 100%. Using the same equation, the classification differences would be zero in all cases and the coefficient of migration would be 0/4 = 0.

It follows that, as the coefficient approaches one, the extent of migration is greater; as the coefficient approaches zero, the extent of migration is less.

Statistical analysis of data

Spearman's rank correlation method was used to determine the correlation, if any, exists between the extent of RSP migration and the increase in the binder solution viscosity[4].

Preparation of RSP tablets

From formula (L), for each binder; the formulations showing the highest and the lowest coefficient of migration, were chosen for sub-sequent compression into tablets. The four layers of each granulation bed were mixed thoroughly before compression. Thus, the prepared formulations are shown in Table (2).

Table 2: RSP tablet batches made using various binder concentrations.

Formula	Lactose granulation specifications		Migration coefficient
	Type and concentration of binder (w/w)	Temperature of drying (°C)	
P1	5 % PVP	50	0.67
P2	15 % PVP	70	0.198
M1	0.5 % MC	50	0.423
M2	1.5 % MC	70	0.089
H1	0.5 % HPMC	50	0.525
H2	1.5 % HPMC	70	0.265
G1	2.5 % gelatin	50	0.467
G2	10 % gelatin	70	0.004

The mixed components of each formula were compressed into tablets using a single-punch tablet machine (12 mm diameter). The tablet weight was adjusted to be 500 mg. Tablets were visually inspected for mottling and evaluated for; uniformity of weight, drug content, uniformity of thickness, friability using Roche friabilator at 25 r.p.m, for 4 minutes, hardness using Erweka hardness tester and disintegration time (USP procedures were used).

***In vitro* release study**

Drug release from the prepared tablets was performed using tablet dissolution apparatus USP I at 50 rpm. The dissolution medium was 100 ml of distilled water, maintained thermostatically at 37±0.5°C. Aliquots of one ml were withdrawn, filtered through millipore filter (0.45 µm), then, diluted with the dissolution medium which is replaced with the same volume of fresh medium to maintain it constant. The released amounts of RSP from each formula were analyzed spectrophotometrically at 266 nm using distilled water as a blank. The experiments were done in triplicates and the mean was calculated.

***In vivo* evaluation**

Tablets prepared with 0.5% w/w MC (batch M1) and those prepared with 10% w/w gelatin (batch G2), were chosen for the *in vivo* study, since they showed the lowest and the highest *in vitro* dissolution rate, respectively. A control batch (identical batch but with no binder) was used for comparison. Six healthy male volunteers were selected for the study. The level of riboflavin in urine was measured after administration of two tablets, (2 mg RSP in each), by using a spectrofluorimetric method[5&6].

Analysis of urine samples

The level of riboflavin into urine was measured by using a spectrofluorimetric method. In this work, the excitation and emission wavelengths were found to be 445 and 520 nm, respectively.

Four ml of urine were introduced into a centrifugation tube and 0.4 ml acetic acid buffer of pH 3.5 was added. The oxidation was started by the addition of 1 ml of $KMnO_4$ solution (4%, w/v). After 1 min, the excess of $KMnO_4$ was reduced by addition of 0.1 ml of H_2O_2 solution (30%). The residue was then extracted by the addition of 4 g of ammonium sulphate and 3 ml of benzyl alcohol saturated with water. The tubes were shaken for 3 min and then centrifuged at 3000 rpm for 5 min. One ml of the supernatant was taken and diluted with 7 ml of a solution containing 45% (v/v) of ethanol in a solution of acetic acid and sodium acetate with a concentration of 0.1 N of each[6]. The samples were then measured spectrofluorimetrically at excitation and emission wavelengths of 445 nm and 520 nm, respectively.

Pharmacokinetic analysis

The prepared batches were compared in terms of urinary recovery of riboflavin during the first 24 hr after administration, maximum urinary excretion rate (R_{max}), and the time (T_{max}) required to reach R_{max}. All parameters were determined from the individual urinary excretion rate–time curves, a plot of urinary excretion rate against the mid-point of urine collection interval.

RESULTS AND DISCUSSION

The viscosity values for the prepared binder solutions are illustrated in Table (3). Following wet granulation and drying of the two formulations (L and C) containing RSP, it was found that, the granules were no longer uniform as to RSP content. Migration of the drug substance had occurred during drying.

Table 3: The viscosity values of binder solutions in different concentrations.

Binder type	Binder concentration (% w/w)	Viscosity (mPa.s)
PVP	5	6.44
	10	11.5
	15	17.44
MC	0.5	15.6
	1	24.9
	1.5	69.8
HPMC	0.5	16.1
	1	35.7
	1.5	72.5
Gelatin	2.5	10.5
	5	13.9
	10	66.55

RSP, being water soluble, followed the solvent as it moved upward and downward during the drying period. Higher concentrations of the drug were found in the outer layers, with a reduced amount toward the center as compared with the initial blended material.

Table (4) shows that, for a specific binder, the values of migration coefficient had a descending order, on increasing the binder solution concentration or viscosity. For formula (L), dried at 50°C, the values of migration coefficient were 0.67, 0.418 and 0.291 for granules prepared with 5, 10 and 15% w/w PVP, respectively. For MC, the values of migration coefficient were 0.423, 0.265 and 0.188 for granules prepared with 0.5, 1 and 1.5% w/w MC, respectively. For HPMC, the values of migration coefficient were 0.525, 0.375 and 0.275 for granules prepared with 0.5, 1 and 1.5% w/w HPMC, respectively. While for gelatin, the values of migration coefficient were found to be 0.467, 0.098 and 0.008 for granules prepared with 2.5, 5 and 10% w/w gelatin, respectively.

So, there is an obvious decrease in the values of the coefficient of migration with increasing the binder concentration and hence viscosity. These results are in agreement with that reported by Warren and Price, (1977 b)[4], who proved that, drug migration decreased with increased binder solution viscosity, where increasing the concentration and therefore, the viscosity of PVP solution has been shown to slow the

migration of propoxyphene hydrochloride in fixed bed of wet granules. This also was proved by Kiekens *et al.*, (1999)[7], who conclud-ed that, the intragranular migration decreased as the granulating liquid viscosity of povidone increased. The viscosity of granulating fluids im-pedes the movement of moisture by increasing the fluid friction[8].

Spearman's rank correlation method was used to determine if a correlation exists between the extent of RSP migration and increasing the binder solution viscosity. The values of r were; - 0.7198, - 0.6264, - 0.6758 and - 0.7363 for formula L (dried at 50ºC), Formula L (dried at 70ºC), formula C (dried at 50ºC) and formula C (dried at 70ºC), respectively, which are considered significantly different than zero. (When r value approaches 1, whatever the sign, there is a good correlation between the two studied variables. While, in case of r value equals zero, there is no correlation.). A significant negative correlation indicates that, there was an inverse relationship between the extent of RSP migration and increasing the binder solution viscosity.

Table (4) reveals that, the migration coefficient and hence the migration of the drug at 70°C is less than its analogue obtained at 50°C.

Newitt & Papadopoulos, (1959)[9] and Pietsch & Rumpf, (1966)[10] postulated that, solute migration on drying is reduced as the drying temperature is raised. They suggested that, at high temperatures, the flow of liquid through the granule cannot maintain the higher rate of drying at periphery and evaporation takes place from progressively further inside the granule. Travers, (1975)[11] stated that,

Table 4: Migration coefficient of RSP using various binders and diluents from granules dried at different temperatures.

Binder type and conc. (%w/w)		Lactose (L)		Calcium phosphate (C)	
		50°C	70°C	50°C	70°C
PVP	5	0.67	0.597	0.475	0.425
	10	0.418	0.337	0.287	0.282
	15	0.291	0.198	0.207	0.143
MC	0.5	0.423	0.286	0.177	0.119
	1	0.265	0.227	0.088	0.089
	1.5	0.188	0.089	0.046	0.024
HPMC	0.5	0.525	0.439	0.253	0.203
	1	0.375	0.336	0.191	0.172
	1.5	0.275	0.265	0.132	0.025
Gelatin	2.5	0.467	0.335	0.133	0.11
	5	0.098	0.076	0.051	0.043
	10	0.008	0.004	0.008	0.0
Water		0.765	0.57	0.602	0.434

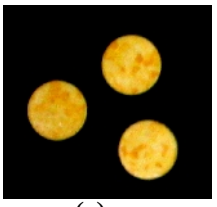
solute migration is likely to be greatest when the evaporation rate is fairly slow at low rates of heat transfer. While, at higher rates, fluid friction will act to retard the moisture movement so that, the granule surface becomes dry at a stage when less overall movement has taken place.

To investigate the effect of the type of major diluent, it is observed from Table (4) that, formula C has a lower coefficient of migration than formula L, i.e. calcium phosphate shows lower migration for RSP than lactose. These results are in agreement with those obtained by Chaudry and King (1972)[12], who assumed that, calcium phosphate had more drug migration inhibitory characteristics than both lactose and calcium sulphate. Also, Stewart *et al*., (1979)[13] reported the adsorption of riboflavin onto water insoluble diluents, including Emcompress (dibasic calcium phosphate). The adsorption of riboflavin onto the surface of Emcompress molecules, may account for the retardation of its migration to the surface of the granulation bed during drying, and hence, lower values of migration coefficient.

Tablet evaluation

Visual inspection of the prepared tablets

Figures (2-5) show different batches of the prepared tablets, exhibiting different degrees of mottling. From Figure (2), it can be observed that, tablets prepared from the granules made with 5% PVP (batch P1), exhibit high degree of mottling, while tablets prepared from the granules made with 15% PVP (batch P2), are somewhat more uniform in color. Figure (3) shows that, tablets prepared from the granules made with 0.5% MC (batch M1), exhibit high degree of mottling. On the other hand, those prepared from the granules made with 1.5% MC (batch M2), are more uniform in color. Figure (4) reveals mottling for batches H1 and H2, (tablets prepared from the granules made with 0.5 and 1.5% HPMC, respectively). Also, Figure (5) shows mottling in tablets prepared from the granules made with 2.5% gelatin (batch G1), while those prepared from the granules made with 10% gelatin (batch G2) are found to be the most homogenously colored ones. Thus, the migration is very clear with the less viscous binder solutions (5% PVP, 0.5% MC, 0.5% HPMC, and 2.5% gelatin), and so contributes for the mottled appearance of the prepared tablets, as observed in batches: P1, M1, H1, and G1. For the higher concentrations of the same binders, it could be observed that, more uniformly colored tablets were obtained. This means that, a more uniform distribution for the drug was obtained upon increasing the binder concentration. This is due to the increase in the viscosity of the binder solutions which in turn, retarded the inter-granular migration of the colored drug into the surface of the granulation bed and thus reduced mottling.



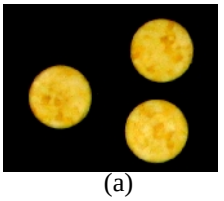
(a)

(b)

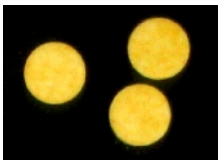
Fig. 2: RSP tablets prepared with PVP K_{25} binder

a- From granules made with concentration 5 % w/w (batch P1).

b- From granules made with concentration 15 % w/w (batch P2).



(a)

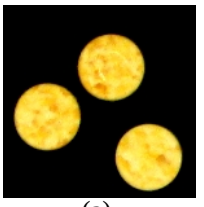


(b)

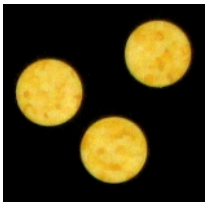
Fig. 3: RSP tablets prepared with MC binder

a- From granules made with concentration 0.5 % w/w (batch M1).

b- From granules made with concentration 1.5 % w/w (batch M2).



(a)

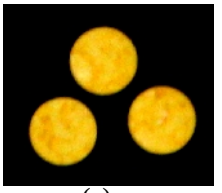


(b)

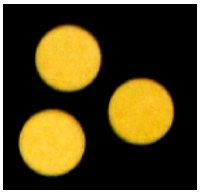
Fig. 4: RSP tablets prepared with HPMC binder

a- From granules made with concentration 0.5 % w/w (batch H1).

b- From granules made with concentration 1.5 % w/w (batch H2).



(a)



(b)

Fig. 5: RSP tablets prepared with gelatin binder

a- From granules made with concentration 2.5 % w/w (batch G1).

b- From granules made with concentration 10 % w/w (batch G2).

Uniformity of weight and thickness

Table (5) reveals that, all the batches of the prepared RSP tablets showing a good uniformity of weight and thickness.

Friability

From the same Table, it could be observed that, batches P2, and G2 comply with the USP XXVII requirements for the friability (% loss does not exceed 1%). Also, it could be observed that, for PVP batches, the percentages of friability were 1.2% and 0.923% for batches of tablets P1, P2 respectively. Thus, increasing the binder concentration from 5% w/w (batch P1) to 15% w/w (batch P2), led to a decrease in friability percentage. These results are in agreement with that obtained by El-Sabbagh *et al*., (1981a)[14], who found that, the increase in the concentration of a specific binder produces more strong granules which on compression, produces tablets of greatest hardness, lowest friability percentages, and highest disintegration time.

However in case of MC, both batches M1 and M2, did not comply with the USP XXVII requirements for the percent friability since the values were > 1% (2.9% and 1.8% for batches M1 and M2, respectively).

Table (5) shows that, the same pattern was obtained for HPMC, with a value of 2.1% and 1.1% for batches H1 and H2, respectively.

In the case of gelatin binder solution, Table (5) shows that, 2.5% w/w concentration produced tablets of 1.93 percent friability (batch G1). On increasing gelatin concentration to 10% w/w, the percent friability was decreased to 0.871% (batch G2).

Hardness

For all binders, the hardness values were increased with the increase in binder concentration, as illustrated in Table (5).

Table 5: Physical properties of riboflavin sodium phosphate tablets, prepared with different concentrations of binders.

Formula	Weight (mg) Mean ± SD	Thickness (mm) Mean ± SD	% Friability	Hardness (Kg) Mean ± SD	Disintegration time (min.)	Dissolution rate (T_{90}) (min.)
P1	496 ± 2.00	2.998 ± 0.021	1.2	5.56 ± 0.625	2.6	10
P2	501.6 ± 1.89	3.036 ± 0.009	0.923	7.93 ±1.1	7.7	15.5
M1	498 ± 2.05	3.034 ± 0.015	2.9	4.5 ± 0.866	1.68	9
M2	498.2 ± 2.2	3.02 ± 0.012	1.8	5.44 ± 0.987	3.9	17
H1	500.2 ± 2.44	3.046 ± 0.011	2.1	4.8 ± 0.629	2.34	8.5
H2	499.9 ± 2.02	3.044 ± 0.011	1.1	6.3 ± 1.02	10.55	37
G1	499 ± 2.28	3.04 ± 0.007	1.93	5.75 ± 0.762	4.07	40
G2	499.6 ± 2.36	3.036 ± 0.019	0.871	7.95 ± 1.2	25	170

Batch M1, (made with 0.5% w/w MC), produced tablets with the lowest hardness values while batch G2, (made with 10% w/w gelatin), produced the highest values within all batches. The high hardness values obtained with gelatin corresponds with its known property of producing hard tablets, as stated by Healy *et al.*, (1974)[15]. The hardness results were parallel and confirm those of friability, i.e. as the hardness increased, the percent of friability decreased and vice versa. Also, these results were in agreement with those obtained by El- Sabbagh *et al.*, (1981b)[16].

Disintegration

From Table (5), it could be observed that, all batches comply with the USP XXVII requirements for disintegration time in distilled water. Increasing the binder concentration is followed by an increase in the disintegration time.

Esezobo and Pilpel, (1976 & 1977)[17&18] confirmed these results. Also, Davies and Gloor, (1972)[19] observed an increase in disintegration time of lactose tablets prepared with higher concentrations of binding agents and they attributed this to the increased binding capacity of these granulating agents at higher concentrations.

Uniformity of drug content

Table (6) reveals that, the range of assay values was the widest in case of batch M1 (which exhibited a considerable drug migration). On the other hand, batch G2 (which exhibited the least drug migration), showed a narrow range of assay values. The high range of assay values may indicate that, the drug is less homogeneously distributed in this batch, and vice versa.

Warren and Price (1977b)[4], proved that, the formula granulated with binder solution of viscosity 3 cps (formula exhibiting considerable drug migration), had an average assay values of 88.1-102.6% of the theoretical amount of drug/tablet. While, the formula granulated with 600 cps binder solution (formula showing little drug migration), had an average assay values of 96.1-102.4% of the theoretical amount of drug/tablet.

Table 6: Riboflavin sodium phosphate content in different tablet batches.

Batch code	Average amount recovered per tablet (mg)	Range of assay values of RSP / tablet (%)
P1	1.756 ± 0.126	77.70 – 96.35
P2	1.826 ± 0.075	84.75 – 97.75
M1	1.754 ± 0.212	74.00 – 108.5
M2	1.878 ±0.069	88.10 – 99.00
H1	1.722 ± 0.129	76.00 – 94.80
H2	1.824 ± 0.142	75.50 – 98.00
G1	1.843 ± 0.109	80.50 – 99.35
G2	1.904 ± 0.055	91.00 – 98.20

***In vitro* drug release**

Results of drug release from different formulations are illustrated in Figures (6-9). Figure (6) shows the effect of PVP concentration on the drug release from different batches prepared with various concentrations of PVP. It could be observed that, batch P1 has a higher dissolution rate than batch P2. This was indicated by the t_{90} value, being 10 and 15.5 min for batches P1 and P2, respectively. Zubair *et al.*, (1988)[20], explained why increasing the binder concentration, increases markedly the disintegration and dissolution times of the tablets. This is because, binders are forced into the interparticular spaces, thereby, increasing the area of contact between particles, leading to the formation of additional solid bonds.

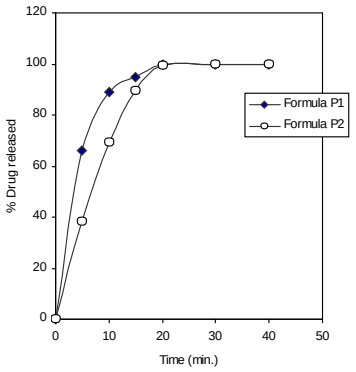


Fig. 6: Effect of PVP concentration on RSP release from its tablets.

It was also suggested that, at lower compression forces, dissolution rate is related to the structure and crushing characteristics of the granules, and, therefore, to the concentration of PVP binder[21].

Chalmers and Elworthy, (1976)[22] noted that, increasing concentration of PVP, resulted in a decrease in the rate of tablet dissolution. They attributed this to the heavier coating of the powder particles with PVP at high concentrations which may act by slowing the rate at which invading water reaches the surface of the powder particles, and slowing diffusion away from the surface of the drug, due to higher viscosity of PVP solutions compared with that of pure water.

Figures (7-9) illustrate the effect of MC, HPMC and gelatin, respectively on the dissolution rate of RSP from tablets. It could be noticed that, the batches prepared with binders of low viscosity (batches M1, H1 and G1) exhibited more rapid dissolution rate (t_{90} = 9, 8.5 and 40 min, respectively), than those prepared with binder solutions of high viscosity (batches M2, H2 and G2 which had t_{90} of 17, 37 and 170 min, respectively).

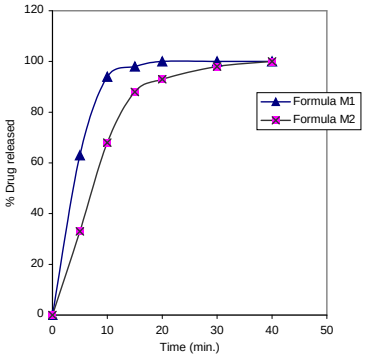


Fig. 7: Effect of MC concentration on RSP release from its tablets.

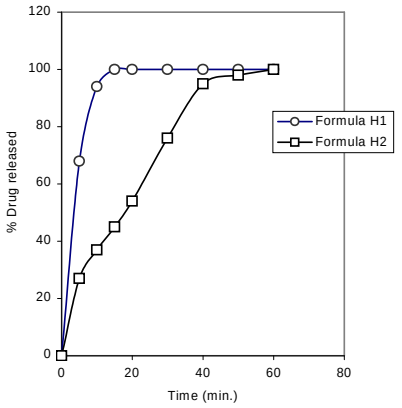


Fig. 8: Effect of HPMC concentration on RSP release from its tablets.

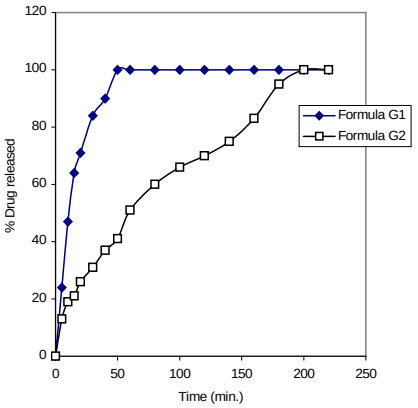


Fig. 9: Effect of gelatin concentration on RSP release from its tablets.

Pharmacokinetic analysis

Figure (10) shows that, tablets prepared with 10% gelatin as a binder (batch G2) exhibited the least amount excreted, while tablets prepared with 0.5% w/w MC (batch M1) and the control batch, prepared with water, showed the highest amount excreted in urine. It could be observed that, there is no difference between M1 and the control batches.

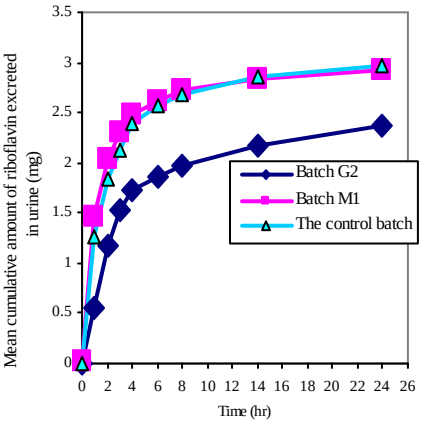


Fig. 10: Average cumulative amount of riboflavin excreted in the urine after oral administration of 4 mg RSP from different tablet batches.

The average excretion rates of riboflavin from different tablet batches are shown in Table (7). The data were analyzed by the analysis of variance test (ANOVA) and the results were illustrated in Table (8). From these Tables, it could be observed that, there is a significant difference between the average excretion rates of the three tested batches at time of 0.5 hr.

Table 7: The average excretion rate * (mg/hr) of riboflavin after oral administration of 4 mg RSP from different tablet batches.

Time (hr)	Excretion rate (mg/hr) (Mean ± SD)		
	Batch (G2)	Batch (M1)	The control batch
0.5	0.546 ± 0.135	1.445 ± 0.299	1.259 ± 0.325
1.5	0.629 ± 0.124	0.569 ± 0.031	0.563 ± 0.143
2.5	0.338 ± 0.067	0.284 ± 0.118	0.303 ± 0.141
3.5	0.198 ± 0.044	0.165 ± 0.095	0.251 ± 0.135
5	0.071 ± 0.027	0.070 ± 0.055	0.091 ± 0.036
7	0.058 ± 0.014	0.049 ± 0.029	0.055 ± 0.020
11	0.032 ± 0.010	0.024 ± 0.026	0.028 ± 0.013
19	0.018 ± 0.009	0.008 ± 0.013	0.011 ± 0.006

• Average of six volunteers

Table 8: Analysis of variance test for the rate of excretion of riboflavin after oral administration of 4 mg RSP from different tablet batches.

Time (hr)	F. value
0.5	19.04 *
1.5	0.6393 •
2.5	0.0353 •
3.5	1.143 •
5	0.509 •
7	0.221 •
11	0.264 •
19	1.642 •

* Extremely significant difference

• Non significant difference among the tested batches by analysis of variance at 5 % level.

In Figure (11), the urinary excretion rate was plotted against the mid-point of a urine collection interval. From this curve, it could be noticed that, the average peaks (R_{max}) were 0.629, 1.445, and 1.259 mg/hr for batches G2, M1, and the control batch, respectively.

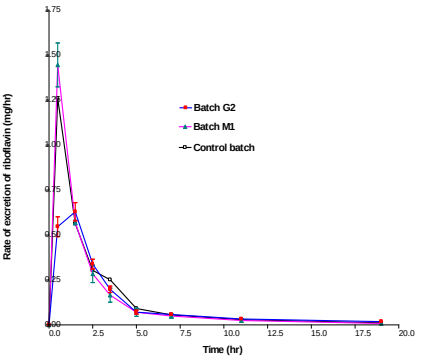


Fig. 11: Average urinary excretion rate of riboflavin after oral administration of 4 mg RSP from different tablet batches.

The values of T_{max} were found to be 1.5 hr for batch G2, and 0.5 hr for both, batch M1 and the control batch. Analysis of data between each two pairs of batches, by student's t- test (Table 9), revealed a significant difference between batches G2 and M1, as well as between batches G2 and the control batch for the value of R_{max}. On the other hand, no significant difference was found between batches M1 and the control batch for the value of R_{max}. These results emphasize that, formulation changes in a drug product may affect the rate of drug bioavailability. The nature and quantity of the binder employed has a profound effect on the bioavailability of the drug from the tablet dosage form. The value of T_{max} for batch G2 was more than its analogues for both, batch M1 and the control batch. This may be attributed to the difference in the dissolution rate of the tested batches, where, the values of $T_{90\%}$ were; 170 and 9 min for G2 and M1 batches, respectively.

Table 9: Statistical t-test for the maximum excretion rate (R_{max}) of riboflavin for G2, M1 and control batches.

The two compared batches	Value of t
Batch G2 and the control batch	4.442 *
Batch M1 and the control batch	1.030
Batch G2 and batch M1	6.188 **

* Highly significant
** Extremely significant
T value critical at 10 degree of freedom and 95% confidence level is 2.23.

On studying the bioavailability of riboflavin, Khalil *et al.*, (1991)[23], found that, tablets belonging to the brand that contained gelatin in the subcoat exhibited poorer dissolution profiles than the other brand containing no gelatin.

Conclusion

From the previous work, it could be concluded that;

- Migration of riboflavin sodium phosphate as an example of colored, low dose, water soluble drug with the solvent during drying of wet granules results in, an uneven distribution of the drug substance within the granulation bed.
- Increasing the binder solution viscosity, decreases the inter-granular migration of the tested drug.
- Riboflavin sodium phosphate mostly showed higher migration at a drying temperature of 50°C than 70°C.
- The type of major diluent employed has a profound effect on the drug migration, where dibasic calcium phosphate showed lower migration than lactose for riboflavin sodium phosphate.
- Mottling is extensively observed for batches prepared with low viscosity binder solutions, while, it diminishes on using high viscous binder solutions.
- Using 10% w/w gelatin, as a binder in the wet granulation method, and 70ºC as the drying temperature of the wet granules, minimized the migration of the studied drug, a case which led to homogenous

distribution of the drug in the prepared tablets which also provoked almost no mottling.

- Tablets prepared with 10% w/w gelatin showed the most uniform drug distribution, but had slower dissolution rate, and higher disintegration time, compared to the other batches.
- The *in vivo* study revealed a lower excretion rate, higher T_{max} and good correlation with the *in vitro* data for riboflavin sodium phosphate tablets prepared with 10% gelatin (batch G2). However, tablets prepared with 0.5% w/w MC (Batch M1) exhibited higher excretion rate than batch G2.

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