Preparation and evaluation of oral capsules containing apigenin nanocrystals prepared by ultrasonication

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Article Info:

Received 17 Mar 2021
Accepted 23 Aug 2021
Published 1 Sep 2021

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Abstract:

This work aims to enhance dissolution rate, increase absorption and bioavailability of Apigenin by formulating it as nanocrystal suspension employing ultrasonic technology. Two different diluents; (5%, 10%) microcrystalline cellulose (MCC) and (2%, 3%) anhydrous lactose were used to prepare hard gelatin capsules for two optimum types for apigenin nanocrystals prepared in our laboratory utilizing ultrasonication technique using 1% tween 80 (F6) and 2% poloxamer 188 (F20). The results showed that the marketed capsules (containing MCC) had about half the dissolution rate than all the prepared nanocrystals capsules formulas, and the nanocrystals prepared with poloxamer gave 90% release within 20 minutes and 100% release with 2 hours with excellent flow properties with no effect of the added diluents while the addition of diluents improved significantly the release of nanocrystals capsules (F6) with 1% tween 80 with good flow properties. The results suggested that utilizing apigenin nanocrystals prepared by ultrasonication technique may improve drug absorption and bioavailability with a reduced required dose.

Key words: Nanocrystals, Apigenin, Ultrasonication, Capsules

تحضير و تقييم كبسول فموي يحتوي على ابيجنن نانو كريستال محضر بالموجات فوق الصوتية

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الخلاصة:

تم استخدام مادتين مخففتين مختلفيتين)5%, 10%(بلورات السليلولوز الدقيقه و) 2%,3%(اللاكتوز اللامائي لتحضير كبسولات جيلاتينيه صلبه لنوعين مثاليين من البلورات النانويه للابجينين المحضره في مختبرنا باستخدام تقنية الموجات فوق الصوتيه باستخدام 1% توين 80 و 2% بولوكسامير 188. واظهرت النتائج ان الكبسولات المسوقه) التي تحتوي (بها حوالي نصف الكميه معدل الذوبان من جميع صيغ كبسولات البلورات النانويه المحضره, واعطت البلورات المحضره باستخدام بولاكسامير 188 اطلاق 90% خلال 20 دقيقه وتحرر 100% مع ساعتين مع خصائص تدفق ممتازه مع عدم وجود تاثير للمخففات المضافه بينما ادت اضافة المخففات الى تحسين كبير في اطلاق كبسولات بلوريه نانويه) اف 6 (مع 1% توين 80 مع خصائص تدفق جيده .اشارت النتائج الى ان استخدام البلورات النانويه مع الابجنين المحضره بتقنية الموجات فوق الصوتيه قد يحسن امتصاص الدواء والتوافر البيلوجي مع تقليل الجرعه المطلوبه.

الكلمات المفتاحية: نانوكريستال، ابيجنن، التراسونيكيشن، كبسول.

Introduction:

The capsule dosage form:

The word 'capsule' in the English language is derived from the Latin word 'Capsule, which means a small box or container. The capsule has been used primarily to describe a solid oral dosage form, which consists of a container, usually made of gelatin. Capsules offer many advantages including, they are easy to swallow, the flexibility of formulation, offers rapid release characteristics, due to the rapid dissolution rate of the capsules, a much shorter process compared to that for other modern dosage forms (e.g., tablet) and controlled release can be achieved using capsules [1].

Dry powder mixtures, granules, pellets, and tablets can be filled into hard capsules. There are three types of excipients used in powder-filled capsules diluents, glidants, and lubricants [2]. The diluents can be defined as inert materials added to a mixture to increase its bulk volume and play a role in the release of active ingredients such as starch, lactose, and microcrystalline cellulose [3]. Glidants are materials used to improve the flowability of powder through the reduction of inter particulate friction such as colloidal and anhydrous silica [4]. Lubricants are materials used to reduce the adhesion between powder and machine used such as magnesium stearate and they are not needed when simple capsule filling equipment is used [5].

Apigenin (AP), 5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1- benzopyran-4- one, is one of the active ingredients found naturally in a variety of plants, fruits, and vegetables, which is usually marketed as dietary supplements [6]. Many pharmacological activities of apigenin have been recognized, including free radical scavenging effects [7], anti-inflammatory effects [8], anti-diabetic effect [9], and growth inhibitory properties in several cancer [10].

Apigenin was classified as a BCS class II drug with low aqueous solubility and high permeability in the intestine. For such poorly soluble compounds, poor solubility would result in a slow dissolution led to poor oral bioavailability and erratic absorption [11].

Some recent works on apigenin were done to enhance solubility and absorption of Apigenin including solid dispersion using poloxamer 127 as micelle forming polymer. The spray drying method was used to prepare solid dispersion of Apigenin-poloxamer 127. The results showed that Apigenin solid dispersion has higher saturated solubility and bioavailability as Cmax was found to be 5 times higher for spray-dried than for non-dried materials [12]. The liquid antisolvent precipitation technique is another method to increase the solubility of Apigenin in ethanol using HP-βCD) as an inclusion complexing agent. The results showed that the Apigenin dissolution rate increased by 68.7 times that of pure Apigenin [13]. Finally, the preparation of Apigenin as nanocrystals using a supercritical antisolvent process result in a rapid dissolution rate compared with coarse powder [14].

The present study aims to prepare and evaluate oral hard gelatin capsules for two types of apigenin nanocrystals prepared in our laboratory using polymeric and surfactant as stabilizers and study the effect of adding different diluents on the flow properties and dissolution profile of the prepared capsules in comparison to market capsules and the pure nanocrystals to select the best oral capsule that may improve the dissolution rate leading to improved absorption and bioavailability.

Materials:

Apigenin (purity > 99.0%) was provided by Hyperchem, china, microcrystalline cellulose (MCC) (Himedia Laboratory, India), anhydrous lactose (Himedia Laboratory, India), hard gelatin capsules (Size 4; Colour: clear), and Apigenin® (SWANSON).

Preparation of capsule dosage forms for the optimum apigenin nanocrystals:

Twenty-four formulas of apigenin nanocrystals were prepared in our laboratory by ultrasonication technique where 500 mg of apigenin powder was dispersed in 10 ml deionized water in the presence of different percentages of tween 80 (1% and 2% w/w) and different percentages of poloxamer 188 (1% and 2% w/w). The samples were placed in a magnetic stirrer at 100 rpm and 25°C for 5 minutes. An ultrasonic system with a probe diameter of 6 mm immersed about 1 cm in the solution with the frequency of 20 kHz. After ultrasonication, apigenin nanosuspension is centrifugated for 15 minutes and filtered and the wet residue was dried at room temperature to receive the final powder and sieved. Two-optimum formulas were selected, the first one (F6) containing 1% tween 80 and the second one (F20) containing 2% poloxamer 188 prepared by different sonication power (600 w and 200 w) respectively, keeping the same sonication time (6 min). To each formula, two types of diluents (widely used) in two different amounts were added to the capsule of apigenin nanocrystals only including 5%, 10% microcrystalline cellulose (MCC), and 2%, 3% anhydrous lactose as shown in table (1). The content was mixed and filled in capsule size 4(1,16).

Table (1): Contents of different prepared apigenin nanocrystal capsules (F6 and F20).

Formulas number	Apigenin nanocrystal	Microcrystalline	Anhydrous lactose
F6a	50	5 (9.1%)	
F6b	50	2.5 (5%)	
F6c	50		1.5 (3%)
F6d	50		1 (2%)
F20a	50	5 (9.1%)	
F20b	50	2.5 (5%)	
F20c	50		1.5 (3%)
F20d	50		1 (2%)

Flow properties of the capsule powder:

The flow properties of F6 and F20 nanocrystals, as well as the prepared capsule powder in addition to the pure supplied apigenin, were studied using various micrometric parameters including:

A- Determination of angle of repose

The fixed funnel method was used for the determination of the angle of repose of the prepared capsule formulas powder. The height of the funnel was adjusted in a way that the tip of the funnel just touches the apex of the heap of the powder. The powder should flow through the funnel freely into the surface [17]. The height and radius of the mound were measured and the angle of repose was then calculated using the following equation:

$$\tan\theta = h / r$$

θ: Angle of repose

h: height of the powder cone

r: radius of the powder cone

The angle of repose is correlated with the flow property according to USP

parameters, as shown in table (2).

Table (2): Flow property according to the angle of repose [18].

Flow property	The angle of repose θ
Excellent	25 to 30
Good	31 to 35
Fair	36 to 40
Passable	41 to 45
Poor	46 to 55
Very poor	56 to 65
Very very poor	Above 66

Determination of the bulked density and tapped density

Each powder sample was poured in a measuring glass cylinder and the untapped apparent volume (Vo) was measured, then the tapped volume (Vf) was measured after successive 1000 tap cycles in a graduated measuring cylinder, until the volume was constant (19). The bulk and tapped densities were calculated according to the equations below:

Bulk density (ρb) = Wt / Vo

Tapped Density (ρt) = Wt / Vf

Where Wt = weight of the prepared powder.

Determination of the Carr's index and Hausner's ratio

Carr's index and Hausner's ratio were estimated for each powder sample to provide a measure of flow properties. They are calculated using the following equations based on the bulk and tapped densities of the blended powder:

Carr's index (%) = [(ρ tapped – ρ bulk) / ρ tapped] x 100

Hausner's ratio = ρ tapped/ρ bulk [20].

Hausner's ratio and Carr's index were correlated with the flow property of blended powder according to the USP as shown in table (3).

Table (3): Flow property according to Hausner's ratio and Carr's index [21]).

Flow character	Hausner's ratio	Carr's index
Excellent	1 to 1.11	<10
Good	1.12 to 1.18	11 to 15
Fair	1.19 to 1.25	16 to 20
Passable	1.26 to 1.34	21 to 25
Poor	1.35 to 1.45	26 to 31
Very poor	1.46 to 1.59	32 to 37
Very very poor	>1.6	>38

In vitro drug dissolution study:

In-vitro dissolution of the prepared apigenin nanocrystals capsules formulas in comparison with the marketed apigenin as well as F6 and F20 nanocrystal and the received drug was carried out using USP type I (basket type) dissolution test apparatus. Each powder was accurately weighed and put into the capsule.
The capsule was kept in the rotary basket at 50 rpm ± 1. The dissolution media was
900 ml of phosphate buffer (pH 6.8) with 0.5% SLS at 37°C. Samples of 5 ml were withdrawn at specific time intervals (5, 10, 15, 20, 30, 45,60, and 120 minutes) and filtered using a 0.22µm millipore filter paper, then analyzed spectrophotometrically at 336 nm. The withdrawn samples were replaced by a fresh dissolution medium to maintain sink condition (21). Each experiment was tested in triplicate and the mean value was calculated.

Result and discussion:

The prepared apigenin nanocrystals using 1% tween 80 (F6) and 2% poloxamer 188 (F20) were selected as the best formulas prepared by ultrasonication technique in our laboratory as they showed high crystallinity with particle size 88.7 nm and 89 nm for nanocrystals prepared by using polymeric and surfactant stabilizers and better flowability and about 41 times solubility increment in simulated intestinal media and significantly higher dissolution rate (98% within
120 min) in comparison to the pure drug which showed only 20% release.
Table (4) show the flow properties of the prepared capsule formulas in comparison to the prepared nanocrystals (F6, F20) and the supplied apigenin. The results showed that the selected nanocrystals (F6) and the capsule formulas (F6a- F6d) containing F6 with microcrystalline cellulose (MCC)/anhydrous lactose had good flow properties with an angle of repose (>30) and Carr's index (>10) and Hausner's ratio (>1.11). While the selected F20 and the capsule formulas (F20a- F20d) had excellent flow properties. This indicating that the presence of tween 80 as a stabilizer in nanocrystal formulas may not affect the flow properties no matter which commonly used diluents were added and similar results were observed with the formulation of nimodipine nanocrystals for oral
administration [22]. While using polymeric stabilizers (poloxamer 188) improved the
flow properties of the prepared powder samples.

Table (4): Description of the flow properties of the prepared apigenin nanocrystal capsules:

Formulas number	Angle of repose	Carr's index	Hausner ratio
Pure received apigenin	61	36	1.56
F6	32.5	12.24	1.139
F20	31	9.2	1.101
F6a	30.8	11.5	1.129
F6b	31.6	11.9	1.135
F6c	31	11.58	1.13
F6d	31.9	12.1	1.137
F20a	29.4	8.45	1.092
F20b	30	8.73	1.095
F20c	29.7	8.49	1.0927
F20d	30	8.52	1.093

In vitro drug dissolution study of apigenin nanocrystals capsules:

Figure (4) shows the dissolution profile of the prepared capsules in comparison to marketed capsules and the supplied apigenin powder (as received).

The prepared nanocrystals capsules F6 and F20 (without diluents) showed a significant ($p<0.05$) increase in the dissolution rate where F6 nanocrystals gave 80% release within 20 minutes and F20 nanocrystals gave 90% and both gave 100% release after 2 hours while the marketed apigenin crystals (containing MCC as diluent) gave only 38% release within 20 minutes and 50% release after 2 hours.

This indicates that poloxamer 188 (in F20) coated the prepared nanocrystals leading to high stabilization and affect the solubility of the drug and hence its dissolution profile. The effect of poloxamer 188 was significantly higher than tween 80 (in F6 nanocrystals). The same results were observed with poloxamer in the production of valproic acid nanostructured lipid carriers [22].

While the prepared capsule formulas (F6a-F6d) showed 90% release within 20 minutes which is higher than that obtained with F6 nanocrystals, indicating that using MCC or anhydrous lactose as the diluent in these capsules enhanced the dissolution rate of apigenin nanocrystals because microcrystalline cellulose allowed powder mass to break up with slight effect on their solubility in the medium while anhydrous lactose make the powder mass more hydrophilic, enabling it to break up more readily on capsule shell disintegration and due to its role in adsorbing moisture (hygroscopic effect) from the nanocrystal powder[3].

While the addition of the diluents to the prepared F20 nanocrystals (F20a-F20d) gave a similar dissolution profile of F20 (without diluent) indicating that optimum formula for apigenin nanocrystals prepared using ultrasonication technique with 2% poloxamer 188 gave more stabilized nanosized crystals led to enhance solubility/dissolution rate with excellent flow properties without the need to add additives.

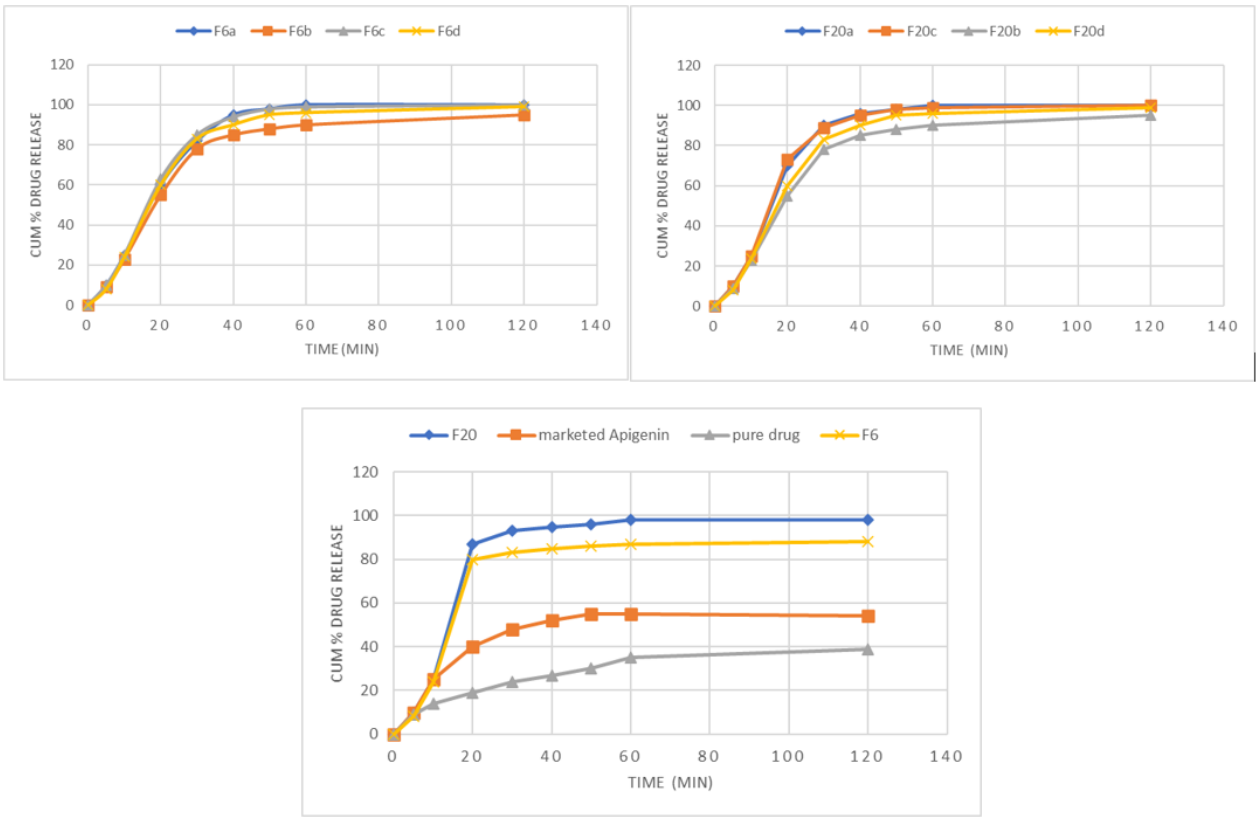

Figure (4): In vitro drug release profile comparison of F6 (a,b,c and d),F20 (a,b,c and d), and apigenin capsules formulas, selected F6 and F20 nanocrystals in comparison to the marketed capsule (Apigenin® SWANSON) and the received drug in pH 6.8.

Conclusion:

This work succeeded to prepare oral hard gelatin capsules for apigenin nanocrystals prepared in our laboratory utilizing the ultrasonication technique. Apigenin nanocrystals powders (F6 and F20) were prepared as apigenin nanocrystals capsules using different diluents showed higher solubility/dissolution profile and excellent flow properties (without additives) while the marketed capsules showed about half the dissolution rate. Indicating that using the ultrasonication technique in the preparation of apigenin nanocrystals significantly enhances apigenin absorption leading to improve its bioavailability and reducing the dose required.

Acknowledgments:

The authors are grateful to Mustansiriyah University (www.uomustansiriyh.edu.iq) Baghdad-Iraq for supporting the present work.

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