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The influence of participation of different apparent density granules on caffeine distribution

Wpływ udziału ziaren granulatów o różnej gęstości pozornej na dystrybucję kofeiny

Granules are preparations consisting of solid, dry aggregates of powder particles with uniform composition, similar size and sufficiently resistant to withstand handling. Granules are presented as single- or multidose preparations, and a semi-product in the formation of tablets as well [7].

Both, the uniformity of composition and parameters of flowing are very important in the production of tablets, influencing the quality of the final product [4]. Bacher et al. [1] examined inhomogeneity of calcium carbonate with different morphologic forms of crystals and sorbitol with different sizes of particles in granules. It was observed that homogeneity of granules increased with a decrease of the size of sorbitol particles.

The aim of the present study was to evaluate the influence of apparent volume and apparent density of two granules on caffeine distribution. Furthermore, spectrophotometric method for determination of caffeine in granules was tested.

MATERIAL AND METHODS

REAGENTS AND INSTRUMENTATION

The following substances were used to prepare granules: anhydrous caffeine (Pharma-Zentrale GmbH, Herdecke, Germany), potato starch (Nowamyl SA Lobez, Poland), lactose (PPH Galfarm Cracow, Poland), gelatin from bovine skin (Sigma Chemical Co. St. Luis, USA), zinc oxide (PPH POCH SA Gliwice, Poland) and glycerol (PPH POCH SA Gliwice, Poland). Hydrochloric acid 0.1 mol/l was used to prepare solutions of caffeine for spectrophotometric examinations (PPH POCH SA Gliwice, Poland).

The patent apparatus for the measurement of repose of powders and granules [5] and modified apparatus for measurement of angle of repose [2] were applied to measure mechanical qualities of granules. The amount of caffeine in solutions obtained from granules was examined in spectrophotometer UV-Vis (Helios Omega Thermo Scientific, USA) with Vision Pro software.

PREPARATION OF GRANULES

The Simple granule with caffeine (GSC).

The formula: caffeine 1.0 parts and Simple granule with the following composition: potato starch 69.3 parts, lactose 29.7 parts, glycerol 86% 2.0 parts, gelatin mucilage 4% (w/w) 98.0 parts.

Preparation: Simple granule prepared earlier and caffeine were weighed out. The granule was rubbed to obtain powder. The caffeine was rubbed in another mortar. The powdered granule was added to caffeine in portions and then the powders were mixed. Distilled water was added in small portions to powders, till the plastic mass was obtained, which was granulated handly through a sieve of 1.02 mm. The granule was dried at room temperature for 24 h. Then, it was sifted trough a sieve of 1.02 mm to obtain uniform granule. The dust was sifted through a sieve of 0.75 mm and these particles were granulated again.

The granule with zinc oxide and caffeine (GZnC).

The formula: caffeine 1.0 parts, zinc oxide 59.4 parts, Simple granule 39.6 parts, gelatin mucilage 6% 30.0 parts. Preparation: Simple granule was rubbed in a mortar, zinc oxide was added and mixed carefully. The powder prepared in this way was added in portions to caffeine that was rubbed earlier. The powder was moistened with gelatin mucilage to obtain a plastic mass, which was granulated handly through a sieve of 1.02 mm. The granule was dried at room temperature for 24 h. Then, it was sifted trough a sieve of 1.02 mm to obtain uniform granule. The dust was sifted through a sieve of 0.75 mm and these particles were granulated again.

MEASUREMENTS OF MECHANICAL PROPERTIES OF GRANULES

Measurement of the apparent volume.

The graduated cylinder of 250 ml was used for measurement of apparent volume of both type of granules before and after settling. The 250 ml of examined granules that is regarded as the unsettled apparent volume was placed into the dry cylinder of the apparatus without compacting. The apparent volume after settling was read after carrying out 10, 250, 500 and 1250 taps from a height of 3±0.2 mm of the cylinder. Such measurements are recommended by The Polish Pharmacopoeia VIII [8].

Measurement of the flow time.

The patent apparatus for the measurement of repose of powders and granules was used for this measurement [5]. During measurement the definite volume of granules (250 ml) was deposited in the funnel of the apparatus and the time of monotonous flow of the whole quantity of granules was measured with a stopwatch. The nozzles under a funnel of the apparatus were marked with Roman numbers which correspond to their diameter in mm.

Measurement of the angle of repose.

The measurement of the angle of repose in the patent apparatus. The measured volume of granule (100 ml) was placed in the funnel of the apparatus with a closed nozzle. After opening of the nozzle the granule was piling up the cone. The height of the cone was measured at constant diameter (9 cm) of its basis, which was marked with the external edge of the apparatus. On the basis of the height of the cone and its diameter, tg of the angle created by freely flowing granule was counted using trigonometrical functions. The measurement was made at constant distance among the funnel and the basis of apparatus (6 cm), size of the nozzle of the funnel (X – 10 mm) and constant volume of examinated granule [5].

The measurement of the angle of repose of granule in the modified apparatus. The apparatus of our own construction was made from Plexiglas and had a cuboid shape [2]. The upper wall of the device was replaced by a movable plate that allowed for free pouring of granule through the narrow nozzle between three side walls. The upper plate is located under the angle of 20°. The granule forms a wedge-shaped block with triangle side walls inside the apparatus. Based on the measurement of height and basis of this triangle it was possible to evaluate the value of the granule angle of repose. The volume of granules was constant (500 ml) [2].

The measurement of volume and mass of particular parts of wedge-shaped block of granule in the modified apparatus.

The 500 ml volume of the granule was measured in the volumetric cylinder and weighed. Then the granule was poured into the apparatus and the wedge-shaped block of granule was divided into 5 fractions with vertical valves putted every 3 cm. Every fraction was precisely weighed and their volume was measured with the volumetric cylinder.

Determination of absorption spectrum and standarization curve for caffeine solution.

The solution of caffeine in hydrochloric acid (0.1 mol/l) with the concentration of 0.01 mg/ml was prepared according to Polish Pharmacopoeia VI. It was measured spectrophotometrically over the range 230 nm to 360 nm. The absorption spectrum of solution reached the maximum at 273 nm [6] (Fig. 1).

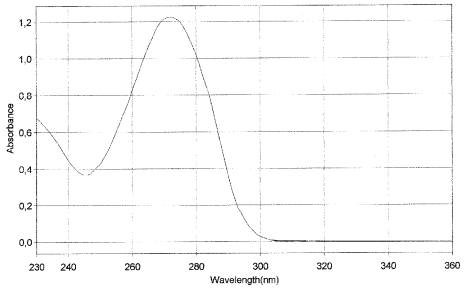

Fig. 1. The absorption spectrum for caffeine solution in hydrochloric acid 0.1 mol/l

Then the absorbance of caffeine solutions in hydrochloric acid (0.1 mol/l) with the concentrations from 0.005 mg/ml to 0.05 mg/ml was measured at maxium λ = 273 nm and the standarization curve was made (Fig. 2).

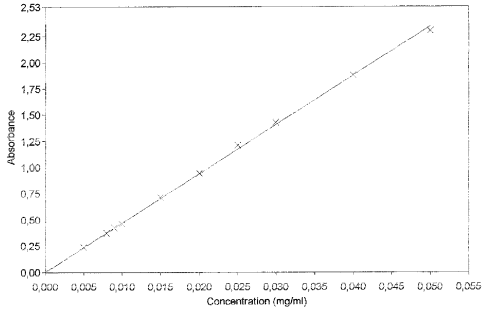

Fig. 2. The standarization curve for caffeine solution in hydrochloric acid 0.1 mol/l

The standarization curve can be described by the equation: **A = 46.9084 x C (**A – absorbance, C – concentration). Coefficient was 0.999074.

The method of determination of caffeine in granule.

The solutions of GS and GZn in 0.1 mol/l HCl were prepared. The absorbance of these solutions was measured at λ = 273 nm. 0.1 mol/l HCl was used as a reference. It was noticed that excipients did not interfere with measurement of caffeine absorbance. The modified apparatus were applied. The granules were divided into 5 fractions every time, and samples weighing 2 g were collected and crushed in a mortar. The sample of 1 g was taken and dissolved in 0.1 mol/l HCl – a bulb was filled

with the acid to 100 ml. Then 1 ml of the solution was taken and diluted with the acid to 10 ml. The concentration of caffeine was measured spectrophotometrically in the solution prepared in that way. The measurement was repeated 6 times for both granules.

RESULTS AND DISCUSSION

The analysis of the data from the examination of granules allowed to define the influence of apparent density of ingredients of granules (zinc oxide, lactose, potato starch) on the properties of their flowing and uniformity of dosage of the active substance.

The apparent volume was finally changed for both granules after 500 taps of cylinder. The difference between apparent volume before and after settling for GSC was 19 ml, whereas for GZnC – 15 ml (Table 1).

Table 1. The apparent volume, ml of granules (n=6)

Number of taps	Apparent volume of GSC	Apparent volume of GZnC
0	250.00	250.00
10	244.33	246.00
250	235.00	237.33
500	231.00	235.00
1250	231.00	235.00

Changes of volume after settling was smaller for GZnC than for GSC. This would be due to differences of densities for both granules. The proper density of GZnC was grater and thereby the granule has greater hardness and less crushing during examination. The GSC had less proper density and larger fragility in comparison to GZnC. It went under crushing and regrupment in a larger rate during the examination and showed larger changes of volume. Knowing masses of particular volumes of granules, the apparent densities were counted (Table 2).

Table 2. Bulk density of granules, g/ml (n=6)

Granule	Density before settling	Density afer settling
GSC	0.4510	0.4877
GZnC	0.7220	0.7677

Density after settling increased in both granules (Table 1 and 2). The GZnC showed larger densities before and after settling than GSC. The measurement of apparent volume allowed calculation of the Hausner ratio and the compressibility index (Table 3). Both of them were assessed from density or volume before and after settling.

Table 3. The Hausner ratio and the compressibility index of granules (n=6)

Granule	Hausner ratio	Compressibility index (%)
GSC	1.08	7.53
GZnC	1.06	5.95

The values of the Hausner ratio for both granules ranged from 1.00 to 1.11, and the compressibility index from 1 to 10%. Parameters of granules indicate very good values of flowing; however, both Hausner ratio and compressibility index were better for GZnC.

Both of the granules flowed only by nozzles VIII–8mm, X–10mm and XII–12mm. With the reduction of diameter of a nozzle under a funnel the time of flow decreased. The GSC had shorter times of flow than GZnC (Fig. 3).

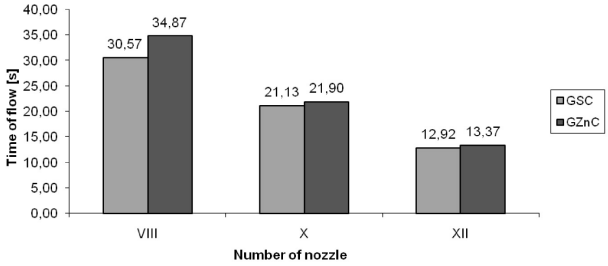

Fig. 3. Association of flow time and type of granules

The values of the angle of repose were greater in patent apparatus than in a modified one (Table 4).

Table 4. The angle of repose α [º] of granules (n=10)

Granule	Patent apparatus		Modified apparatus	
	tg α	α (º)	tg α	α (º)
GSC	0.78	38.09	0.71	35.25
GZnC	0.87	40.91	0.72	35.86

It could be associated with different conditions of measurement. There was only 100 ml of granule in the funnel of patent apparatus and 500 ml in a modified one. A greater amount of granule used in second case could cause stronger sinking of its particles. The pressure of next portions of granule could bring about a decrease of the formed angle. Another explanation is a different structure of the apparatus. In a patent apparatus there was a base with a round external border which limits spilling of granule and assured constant diameter of the base of a cone of the measured material. But this base was significantly smaller than that formed by a granule in a modified apparatus. The GSC had a smaller angle of repose than GZnC. Data of time of flow and angle of repose indicated that GSC had better properties of flowing (smaller angle of repose and shorter time of flow) than GZnC.

The apparent density of separate fractions of granules was also evaluated in the modified apparatus. Then the content of caffeine in each fraction was measured spectophotometrically (Table 5) and the obtained results were validated (Tables 6–8).

CONCLUSIONS

The results of the study indicated that GSC had smaller apparent density of particles as compared to GZnC. There were no big differences between apparent density in separate fractions. Mean density for GSC was 0.4582 g/ml and for GZnC was 0.7460 g/ml. We could observe a similar dependence in the content of caffeine in particular parts of cone formed by the granules. This was proved by spectrophotometric measurements. Mean content of caffeine in GSC was 4.4383 mg/ml and in GZnC 6.3103 mg/ml. There was a similar level of it in all volumes of granules. That means that the content of caffeine increases with an increase of density of granules and is still in particular fractions in the same product.

Table 5. Measured and calculated data for each fraction of granules (n=6)

Granule	No of granule fraction	Mass of granule M_g (g)	Volume of granule V (ml)	Density of granule d (g/ml)	Absorbance A	Concentration of caffeine in examined solution C (mg/ml)	Mass of caffeine in 1 g of granule M_{cof} (mg/g)	Volume of 1 g of granule V=1/d (ml/g)	**Mass of caffeine in 1 ml of granule C_{cof} (mg/ml)**	Mass of caffeine in granule fraction $M_f = M_g \times M_{cof}$ (mg)
GSC	I	67.7370	147.0000	0.4614	0.4560	0.0097	9.7211	2.1702	**4.4854**	658.3027
	II	60.2802	132.1667	0.4561	0.4395	0.0094	9.3693	2.1927	**4.2731**	564.3479
	III	48.4678	106.1667	0.4566	0.4610	0.0098	9.8277	2.1902	**4.4876**	475.7105
	IV	30.9512	66.0000	0.4691	0.4513	0.0096	9.6216	2.1327	**4.5125**	297.8215
	V	14.2640	31.8333	0.4478	0.4647	0.0099	9.9058	2.2348	**4.4327**	140.9094
GZnC	I	122.4655	162.3333	0.7543	0.3918	0.0084	8.3532	1.3261	**6.2988**	1022.6282
	II	101.1333	134.8333	0.7517	0.3827	0.0082	8.1577	1.3309	**6.1330**	822.3560
	III	67.9302	91.0000	0.7488	0.4132	0.0088	8.8079	1.3361	**6.6029**	593.8104
	IV	44.5107	58.5000	0.7619	0.3975	0.0085	8.4740	1.3139	**6.4542**	376.3864
	V	23.9020	33.5000	0.7134	0.3988	0.0085	8.5024	1.4028	**6.0626**	203.3758

Table 6. Validation of measurements of mass, volume and absorbance

Granule	Granule fraction	Validated quanities	n	min	max	M	SD	SE	CV%
GSC	I	M	6	64.1590	74.6040	67.7370	3.8693	1.5796	5.7122
		V	6	133.0000	163.0000	147.0000	10.0399	4.0988	6.8299
		A	6	0.4230	0.4840	0.4560	0.0242	0.0099	5.3068
	II	M	6	54.8180	65.1000	60.2802	3.4797	1.4206	5.7726
		V	6	120.0000	142.0000	132.1667	7.5476	3.0813	5.7107
		A	6	0.3900	0.4640	0.4395	0.0262	0.0107	5.9677
	III	M	6	44.5040	51.3070	48.4678	2.6117	1.0662	5.3885
		V	6	98.0000	112.0000	106.1667	6.1128	2.4956	5.7578
		A	6	0.4360	0.5000	0.4610	0.0258	0.0105	5.6014
	IV	M	6	29.3300	33.0340	30.9512	1.3391	0.5467	4.3264
		V	6	62.0000	70.0000	66.0000	2.8983	1.1832	4.3913
		A	6	0.4280	0.4680	0.4513	0.0144	0.0059	3.1944
	V	M	6	10.8320	16.2080	14.2640	2.6647	1.0878	18.6811
		V	6	24.0000	37.0000	31.8333	5.7764	2.3582	18.1457
		A	6	0.4340	0.4770	0.4647	0.0167	0.0068	3.5925
GZnC	I	M	6	111.5160	138.1180	122.4655	9.1662	3.7421	7.4847
		V	6	152.0000	184.0000	162.3333	11.4833	4.6880	7.0739
		A	6	0.3740	0.4120	0.3918	0.0135	0.0055	3.4370
	II	M	6	87.2650	115.2370	101.1333	11.0999	4.5315	10.9755
		V	6	112.0000	158.0000	134.8333	17.4174	7.1106	12.9177
		A	6	0.3370	0.4020	0.3827	0.0261	0.0106	6.8098
	III	M	6	56.1150	79.9000	67.9302	8.9040	3.6350	13.1076
		V	6	73.0000	110.0000	91.0000	14.1421	5.7735	15.5408
		A	6	0.3510	0.5280	0.4132	0.0607	0.0248	14.6796
	IV	M	6	41.5430	48.7170	44.5107	3.0492	1.2448	6.8505
		V	6	54.0000	64.0000	58.5000	4.8062	1.9621	8.2158
		A	6	0.3650	0.4210	0.3975	0.0195	0.0079	4.8982
	V	M	6	20.3530	31.2860	23.9020	4.3110	1.7600	18.0361
		V	6	29.0000	44.0000	33.5000	5.9582	2.4324	17.7856
		A	6	0.3770	0.4300	0.3988	0.0186	0.0076	4.6650

M – mass, V – volume, A – absorbance, n – size of test, min and max – range of value, M – arithmetic mean, SD – standard deviation, SE – average standard error, CV% - coefficient of variation

Table 7. Variance analysis for fractions I – V of granules

Validated quanities	GSC		GZnC	
	H	p	H	p
M	27.58	< 0.0001	27.58	< 0.0001
V	27.53	< 0.0001	27.61	< 0.0001
A	3.80	0.4340	1.59	0.8104

M – mass, V – volume, A – absorbance, H – value of test function (for variance analysis), p – significance level

Table 8. Differences of three validated quantities between fractions I – V of GSC vs GZNC

Validated quanities	I GSC vs GZNC	II GSC vs GZNC	III GSC vs GZNC	IV GSC vs GZNC	V GSC vs GZNC
M	0.0039	0.0039	0.0039	0.0039	0.0039
V	0.0250	0.6889	0.0374	0.0163	0.8102
A	0.0039	0.0250	0.0547	0.0039	0.0039

M – mass, V – volume, A – absorbance

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SUMMARY

Granules are preparations consisting of solid, dry aggregates of powder particles with uniform composition, similar size and sufficiently resistant to withstand handling. Granules are presented as single-dose or multidose preparations, and a semi-product in the formation of tablets as well. The aim of the study was to evaluate the two granules with different apparent volume with caffeine. The transfer of their particles with the methods indicated by Ph. Eur. 5.0 and Polish Pharmacopoeia VIII was examined. The patent apparatus for the measurement of angle of repose and the time of flow powders and granules and modified apparatus for measurement of angle of repose which enables fractionating of reposed granule were applied. Furthermore, the spectrophotometric method for determination of caffeine in granules, was tested. It allows evaluation of distribution of the active substance in particular fractions of drug form and changes of granules density on distribution of active substance in a reposed granule.

STRESZCZENIE

Granulaty są agregatami sproszkowanych substancji o jednolitym składzie, podobnej wielkości i odpowiedniej odporności mechanicznej. Mogą być zarówno postacią leku jedno- lub wielodawkowego, jak i półproduktem służącym do produkcji tabletek. W pracy otrzymano dwa granulaty o różnej gęstości pozornej, zawierające kofeinę. Zbadano właściwości przemieszczania się ich ziaren metodami podanymi przez Ph. Eur. 5.0 i FP VIII. W tym celu zastosowano aparat patentowy do pomiaru kąta usypu i czasu zsypywania proszków i granulatów oraz zmodyfikowany aparat do pomiaru kąta usypu, który umożliwia frakcjonowanie zsypanego granulatu. Opracowano też spektrofotometryczną metodę oznaczania kofeiny w granulatach, która pozwala na ocenę dystrybucji substancji czynnej w poszczególnych frakcjach postaci leku.