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ASSESSING THE HOMOGENEITY OF ACTIMASK® ACETAMINOPHEN AND N-ACETYL GLUCOSAMINE MIXTURE TO PREDICT FURTHER STEPS IN THE DEVELOPMENT OF ORODISPERSIBLE TABLETS

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Abstract

One of the main problems in the production of tablets, which has significant negative consequences, is the segregation of the tablet mixture leading to inhomogeneity of dosage units, material losses in the manufacturing process and improperness of the specified pharmaceutical technical characteristics of the mixture.

The aim of the research. This work aims at the pharmaceutical technical study of the substances N-acetyl-D-glucosamine and Actimask® Acetaminophen and determination the uniformity of the powder mixture of active pharmaceutical ingredients (APIs) to predict the optimal technology for obtaining a pharmaceutical formulation with the acceptable properties.

Materials and methods. N-acetyl-D-glucosamine (Zhejiang Candorly Pharmaceutical, China) and Actimask® Acetaminophen (SpiPharma, USA) were used.

Scanning probe microscope Solver P47N-PRO («NT-MDT», Russia), optical microscope, flowability tester VP-12A, laser diffraction particle size analyzer SALD-2201 («Shimadzu», Japan), liquid chromatograph Agilent 1260 Infinity II with Diode Array Detector (Agilent Technologies, USA), spectrophotometers Shimadzu UV-1800 («Shimadzu», Japan) were used.

The study of API pharmaceutical technical properties (microscopic characteristics, moisture absorption capacity, flowability, bulk volume and tapped volume, particle size distribution by sieve analysis and laser diffraction), as well as vibration simulation and following chromatographic study were carried out in this work.

Results and discussion. The shape of the particles N-acetyl-D-glucosamine and Actimask® Acetaminophen, which was determined by microscopic analysis, demonstrated the possibility of N-acetyl-D-glucosamine particles to stick to Actimask® Acetaminophen ones. The experimental study allowed to reveal the hygroscopicity of both APIs; poor flowability, unsatisfactory Hausner ratio, and Carr index for N-acetyl-D-glucosamine; excellent flowability, Hausner ratio, and Carr index for Actimask®. Vibration caused segregation of the powder mixture. It was found that all layers do not meet the requirements and an excessive content of Actimask® is registered, which indicates the stratification of the powder mixture.

Conclusions. The physical properties of the substances were determined and found to have significant differences in their particle size distribution. Segregation of the mixture after vibration was confirmed by laser diffraction and assay analysis. In order to solve the segregation problem, the granulation of N-acetyl-D-glucosamine may be proposed.

Keywords: N-acetyl-D-glucosamine, Acetaminophen, segregation, laser diffraction, orodispersible tablets, direct pressing, mixture uniformity, HPLC.

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1. Introduction

Orally Disintegrating Tablets (hereinafter referred as ODTs) represent a modern direction in the development of solid dosage forms. Due to the rapid disintegration in a small amount of fluid, ODTs are characterized by better bioavailability, ease of administration, and less toxic effects of active pharmaceutical ingredients (APIs) on the liver due to partial alleviation of the first-pass effect.

One of the promising candidates for incorporation into the ODTs is Actimask® Acetaminophen, an antipyretic and analgesic agent characterized by the absence of unpleasant taste sensations due to the gelatin coating applied. According to international recommendations (WHO, Medscape, German Society of general and family medicine, etc.), Acetaminophen is classified as a first-line drug for the relief of chronic pain [1, 2]. It should be noted that in comparison with NSAIDs, also recommended for the treatment of chronic pain syndrome (metamizole, naproxen, ibuprofen, etc.), Acetaminophen is considered to be safer due to fewer side effects, primarily such as gastro- and cardiotoxicity [3, 4]. However, a significant disadvantage of Acetaminophen is hepatotoxicity in high doses and/or with long-term treatment, which can especially occur in the long-term treatment of chronic diseases [5]. A possible approach to reducing or even avoiding the manifestation of Acetaminophen toxicity is its combined use along with N-acetyl-D-glucosamine. According to the literature, the combination of Acetaminophen with N-acetyl-D-glucosamine reduces hepatotoxicity and potentiates the analgesic effect of Acetaminophen [6]. However, the use of two substances that may differ in their physical and chemical properties (primarily morphology and size distribution) increases the risk of segregation of the tablet mixture representing one of the main problems in tablet preparation development and manufacturing process.

Segregation issues can cause improperness of the specified pharmaceutical technical characteristics of the mixture, material losses in the manufacturing process, and inconsistent tablet batches with unacceptable dosage variations. As a result, the amount of quality product created within a single batch can be reduced by up to 70 % of the total batch [7, 8]. In addition, the segregation is responsible for unsatisfactory validation due to the non-reproducibility of the technological process of obtaining the product with an acceptable content of active and auxiliary substances [9, 10].

Ensuring homogenous mixing of the tablet mass components is an important factor for obtaining high-quality products. The ideal mixture has a uniform distribution of all components. Uniformity in the traditional technological sense refers only to the distribution of APIs, provided that the excipients are also uniformly distributed.

The homogenization process occurs in competition with segregation hindering perfect mixing. Segregation can be described as the process of spontaneous separation of powder mixture components that was previously uniformly dispersed with each other [8, 9]. Segregation occurs because during the movement of the powder mixture (which is a component of industrial production) fractions that differ in physical properties, such as particle size, shape, density, surface roughness, etc., manifest themselves differently [10, 11].

The occurrence of segregation is driven by a set of physical and chemical properties of raw materials and production technological features. There are two main mechanisms of segregation in pharmaceutical manufacturing – sieving and fluidization. The first one is regarded as the most common and takes place when there is a large enough size variation of particles in a mixture – typically differences in the mean diameter of 3 times or more. In such case smaller particles move down through larger particles [12, 13]. This mechanism is possible when vibration acts on the powder mixture, for instance, during pouring, tableting, etc. Fluidization segregation can occur when a mixture contains a large portion of light or fluffy free-flowing, fine component and a smaller portion of a relatively heavier component. The larger component easily penetrates the fluidized fines, pushing the fines layer to the top of the bin or vessel [14, 15]. The fine powder particles can also remain suspended in the air stream, for example, when filling a hopper [16].

The ability of tablet mixture to segregate can be examined in several ways. One of them is based on the special devices that detect segregation in a matter of minutes. For instance, SPECTester© material segregation tester, which uses spectroscopic technology and can analyze a sample consisting of up to six individual components, provides a simple report indicating why and how much the mixture is segregated in terms of component concentrations, particle size differences, and product homogeneity [9, 10]. However, such tools may be not available due to their expensiveness.

Another way involves testing the mixtures using the equipment able to simulate vibration and/or fluidization. Various tools are applicable for this purpose. L. Břenkováa et al. used the special device (pipe) made of stainless steel with a lid of length 250 mm, and an inner diameter of 25 mm. Mixture homogeneity testing on a laboratory scale was performed by selecting 5–7 samples along with the axial position of the segregation tube after vibrating the device along the axis (amplitude 40–300 mm, frequency 1–4 Hz) for 10–60 minutes. The quantitative composition was studied by HPLC [17]. M. Jaklič et al. used in their work a transparent pipe that was divided into two parts with a mechanically opening valve between them. The test substance was placed in the upper pipe, the sliding valve was opened and the mixture was poured down. A fluidized bed was formed due to dynamic air from the bottom up. Samples were taken from several layers and the composition was studied by laser diffraction. However, only monocomponent powders were investigated for segregation ability [18].

The aim of this work is to carry out the pharmaceutical technical study of N-acetyl-D-glucosamine and Actimask® Acetaminophen and determine the segregation ability of their mixture in order to substantiate further steps in the development of pharmaceutical preparation with acceptable quality attributes.

2. Materials and methods

N-acetyl-D-glucosamine (Zhejiang Candorly Pharmaceutical, China) and Actimask® Acetaminophen (SpiPharma, USA), which is acetaminophen substance coated with a gelatin shell (active ingredient content is 93.2 %), were used in this study.

Scanning probe microscope Solver P47 N-PRO («NT-MDT», Russia), optical microscope, flowability tester VP-12A, laser diffraction particle size analyzer SALD-2201 («Shimadzu», Japan), liquid chromatograph Agilent 1260 Infinity II with Diode Array Detector (Agilent Technologies, USA), spectrophotometers Shimadzu UV-1800 («Shimadzu», Japan) were used.

2.1. Experimental procedures

Pharmaceutical technical properties were studied according to conventional methods: microscopic analysis, flowability, bulk density, tapped density, Carr index, Hausner ratio, and moisture absorption capacity [19].

Vibration. The vibration effect on the tablet mixture was simulated using a cone funnel with a closed bottom. The model mixture was prepared in the amount of 100 g by mixing Actimask® acetaminophen and N-acetyl-D-glucosamine in a ratio of 4:1, respectively, based on 100 % of active substances. Immediately after mixing the homogeneous mixture was placed in a funnel connected to a vibrating device. After 10 min of vibrating the test samples were taken in three sites of the mixture – upper, middle, and lower (**Fig. 1**). For quantitative determination, the API mixture from each site was compressed into tablets with a nominal weight of 500 mg.

Fig. 1. Sampling sites

Measurement of the size distribution of powder particles by laser diffraction. The particle size and size distribution were determined by laser diffraction (Ph.Eur. 2.9.31). For this test, samples of the mixture before and after vibration were used.

A mixture of *ethyl acetate R* – vaseline oil (1:2) was used as a medium to prepare the dispersion of the powder sample to be analyzed. 50 mg of the powder was placed into a 20 ml volumetric flask and thoroughly mixed with 10 ml of *ethyl acetate R* – vaseline oil (1:2) medium until the

powder particles were completely wetted. Then the volume of the dispersion was brought to the mark and placed in an ultrasonic bath with a power of 50–100 W for 1 min, thoroughly mixed and then the sample was immediately taken for the measurement.

Acetaminophen assay by spectrophotometry. The tests were performed by absorption spectrophotometry in the ultraviolet and visible range according to the requirements of Ph.Eur. 2.2.25.

Test solution. 10 tablets were weighed and the average weight of the tablets was determined. The weighed tablets were powdered and mixed thoroughly. To the precisely weighed amount of powdered tablets equivalent to 150 mg of acetaminophen, 80 ml of *0.01 M sodium hydroxide solution* heated to about 60 °C was added, shaken for 20 min, and cooled. The volume of the solution was adjusted to 250.0 ml with the same solvent, stirred, and filtered through a 13 mm diameter PTFE (hydrophilic) membrane filter with a pore size of 0.45 μm, discarding the first 3 ml of filtrate. The resulting solution was used to prepare a solution with a acetaminophen concentration of 6 μg/ml in *0.01 M sodium hydroxide solution*.

Reference solution. The solution of *acetaminophen pharmacopeial reference standard (PhRS)* in *0.01 M sodium hydroxide solution* with a concentration of 6 μg/ml acetaminophen was prepared.

Compensation solution. 0.01 M sodium hydroxide solution.

The optical density of the test solution and the reference solution was measured at a wavelength of 257 nm relative to the compensation solution.

The content of $C_8H_9NO_2$ in the tablet was calculated based on the average tablet weight and the declared content of $C_8H_9NO_2$ in *acetaminophen PhRS*.

N-acetyl-D-glucosamine assay by high performance liquid chromatography. The tests were performed by liquid chromatography according to the requirements of Ph.Eur. 2.2.29, 2.2.46.

Test solution. 10 tablets were weighed and the average weight of the tablets was determined. The weighed tablets were powdered and mixed thoroughly. To the precisely weighed amount of powdered tablets equivalent to 25 mg of N-acetyl D-glucosamine, 150 ml of *water R* was added and shaken for 20 min. The volume of the solution was adjusted to 250.0 ml with the same solvent, mixed, and filtered through a membrane PTFE (hydrophilic) filter with a diameter of 13 mm and pore size of 0.45 μm, discarding the first 3 ml of filtrate (concentration of N-acetyl D-glucosamine 100 μg/ml).

Reference solution. The solution of *N-acetyl D-glucosamine PhRS* in *water R* with a 100 μg/ml N-acetyl D-glucosamine concentration was prepared.

Phosphate buffer solution pH 6.0. 2,722 g of *potassium dihydrogen phosphate R* was dissolved in 900 ml of *water for chromatography R*. 1 M solution of potassium hydroxide was added to pH 6.0, and then the volume of the resulting solution was adjusted to 1000 ml with *water for chromatography R* and stirred.

The comparison solution and the test solution were chromatographed using a high-performance liquid chromatograph with diode array detector (Agilent Technologies, USA), obtaining the required number of chromatograms, under the following conditions:

– *column:* Spherisorb Amino (NH_2), 80Å (P.N.: PSS832313), size 4.6 mm × 150 mm, with a particle size of 3 μm or similar, which comply with the requirements of the test «Checking the suitability of the chromatographic system»;

– *mobile phase: acetonitrile K1 – phosphate buffer solution pH 6.0* (75:25);

– *column oven temperature*: 25 °C;

– *rate of mobile phase*: 0.6 mL/min;

– *detection wavelength*: 194 nm;

– *injection*: 20 μl;

– *chromatography time*: 2.5 times longer retention time of N-acetyl D-glucosamine.

Suitability of the chromatographic system:

– the number of theoretical plates calculated from the peak of N-acetyl-D-glucosamine on the chromatogram of the reference solution must be at least 600;

– the symmetry factor calculated for the N-acetyl-D-glucosamine peak on the chromatogram of the reference solution must be not less than 0.8 and not more than 1.5;

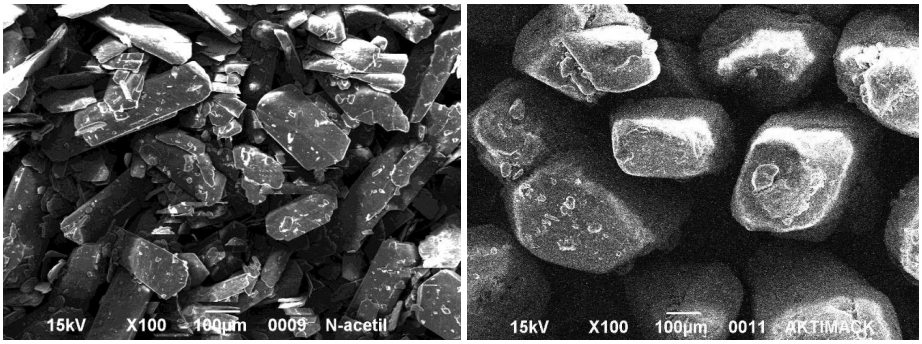
– the convergence calculated for the areas of the N-acetyl-D-glucosamine peaks, on the reference solutions chromatograms, should not exceed the values given in **Table 1**.

Table 1
Critical values of convergence

Number of injections, n_0	Acceptable Relative Standard Deviation (RSD ≤)
2	0.25
3	0.67
4	0.96
5	1.19
6	1.38

3. Results

At the first stage, API microscopy was performed. The shapes of particles and their surface character are determined (**Fig. 2**).



a *b*

Fig. 2. Microscopic characterization of APIs:
a – N-acetyl-D-glucosamine; *b* – Actimask® Acetaminophen

As it can be seen from the micrograph depicted in **Fig. 2, *a***, N-acetyl-D-glucosamine is formed by particles of different sizes, but the same shape (the length is much longer than the width). The particle surface has small roughness, facilitating the adhesion of smaller particles to larger ones.

The shape of Actimask® Acetaminophen particles is more uniform, as compared to N-acetyl-D-glucosamine, and close to spherical (**Fig. 2, *b***). The particle surface has fragmentary small hollows where fine particles stick. From the micrographs, it can be supposed that the particles of the studied substances could form conglomerates due to sticking finer to coarser ones.

The next step included the microscopy of Actimask® – N-acetyl-D-glucosamine combination in a ratio of 4:1 (selected based on the previous pharmacological studies). As it can be seen from the micrograph depicted in **Fig. 3**, small particles of N-acetyl-D-glucosamine are layered on the roughness of the Actimask® surface and form a conglomerate (N-acetyl-D-glucosamine is highlighted white on Actimask® sphere). This indicates the possibility of direct mixing of the APIs to obtain a homogeneous mixture.

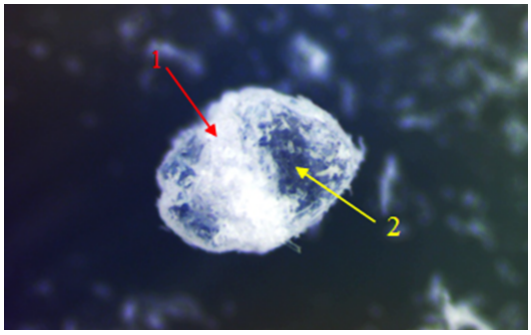


Fig. 3. Microscopic characterization of Actimask® – N-acetyl-D-glucosamine combination (4:1)

The bulk properties of the APIs and their mixture (4:1) are given in **Table 2**.

Table 2

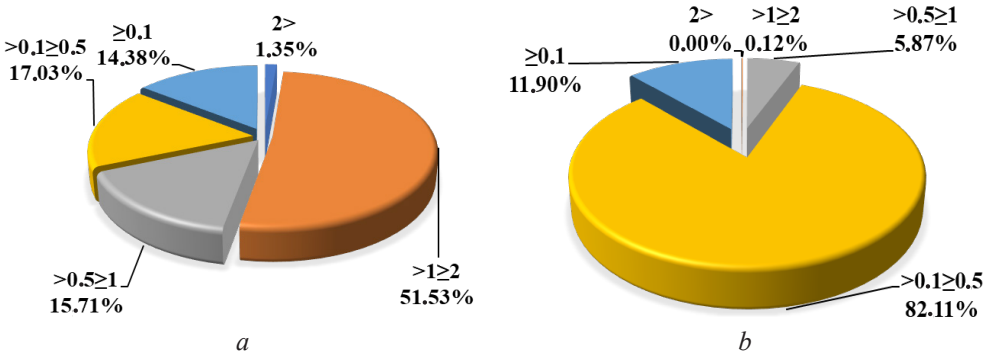
The bulk properties of the APIs and their mixture (4:1)

Index	Test sample		
	Actimask® Acetaminophen	N-acetyl-D-glucosamine	Mixture 4:1
Bulk density, g/ml	0.6913±0.0063	0.4000±0.0032	0.6874±0.0046
Tapped density, g/ml	0.7713±0.0069	0.6238±0.0064	0.8821±0.0076
Flowability, g/s	13.8800±0.0501	0.3226±0.0050	3.9100±0.0035
Carr index, %	10.37	35.87	22.07
Hausner ratio	1.1157	1.5595	1.2832
Angle of repose, °	17.14±0.82	51.00±0,50	27.70±1.07
Flow characteristic	Excellent	Very poor	Passable

Note. n = 5, P = 95 %

As it can be seen from the above data, N-acetyl-D-glucosamine powder is a light, dust-forming substance with insufficient flowability. Actimask® has excellent flowability, suggesting its easy dosing, mixing, and less material loss during the manufacturing process of tablets. Mixing 1 part of N-acetyl-D-glucosamine and 4 parts of Acetaminophen gives a practically homogenous blend. This fact may be explained by coating coarser particles of Acetaminophen with finer particles of N-acetyl-D-glucosamine. The pharmaceutical technical properties of the mixture are better than that of pure N-acetyl-D-glucosamine, and worse than that of Actimask®. However, the combination study suggests that the flowability is satisfactory, and bulk and tapped densities are within acceptable limits.

The percentage of particle size fractions for both APIs was determined by sieve analysis. The results obtained are shown in **Fig. 4**. N-acetyl-D-glucosamine particles are quite different in their sizes, and this fact indicates the heterogeneity of the API (**Fig. 4, *a***). The most percentage of 51.53 % is the fraction with particles from 1 to 2 mm. Actimask® substance appears to be more homogenous in its particle size distribution: the dominant fraction (82.11 %) consists of particles ranging from 0.1 to 0.5 mm (**Fig. 4, *b***). Due to the differences in the particle size distribution of N-acetyl-D-glucosamine and Actimask®, stratification of the mixture is possible during mixture pouring and die filling in direct compression, which, in turn, can affect the uniformity of the APIs in dosage units.



a *b*

Fig. 4. The percentage of particle size fractions for both APIs determined by sieve analysis: *a* – N-acetyl-D-glucosamine; *b* – Actimask® Acetaminophen

A further step was devoted to the determination of moisture absorption capacity of the substances being studied (**Fig. 5**).

The initial moisture contents in APIs were 1.99 and 0.40 % for Actimask® and N-acetyl-D-glucosamine, respectively. Under relative humidity (RH) of 100 %, the Actimask® moisture content raised to 9.74 % per 24 hours, while that of N-acetyl-D-glucosamine reached 4.63 %,

i.e., half as much. Under RH of 40 %, Actimask® and N-acetyl-D-glucosamine absorbed water up to 2.18 % and 0.75 %, respectively. Therefore it is possible to conclude, that the substances are stable under normal humidity levels.

The particle size analysis was also performed by the laser diffraction method. At the first stage, APIs, Actimask® Acetaminophen and N-acetyl-D-glucosamine, were studied separately.

According to **Fig. 6** and **Table 3**, a median size of Actimask® particles is 446.68 µm, while the particle size range is from 301.73 (10 %) to 874.69 (90 %) µm.

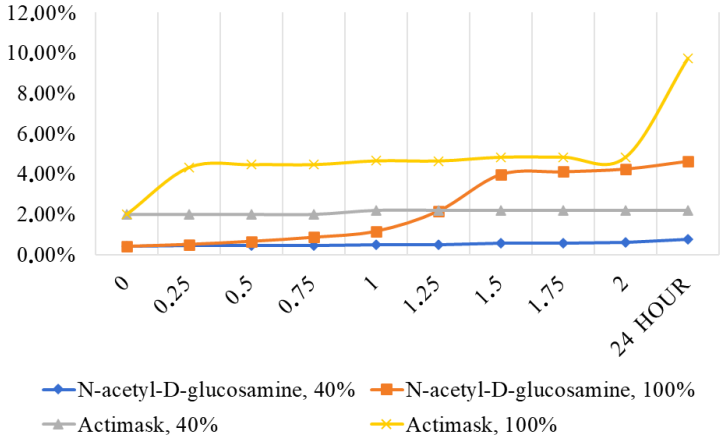


Fig. 5. API moisture absorption capacity study

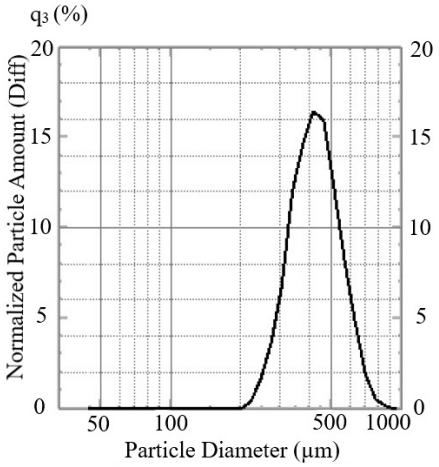


Fig. 6. Actimask® Acetaminophen particle size

Table 3

Actimask® Acetaminophen particle size distribution

Vol. Under	1st measurement	2nd measurement	3rd measurement
10 % D(µm)	301.730	325.811	320.951
20 % D(µm)	336.034	362.610	352.028
30 % D(µm)	363.140	401.555	386.114
40 % D(µm)	392.432	429.708	415.584
50 % D(µm)	419.796	459.835	443.147
60 % D(µm)	448.033	492.075	472.538
70 % D(µm)	478.169	534.454	505.302
80 % D(µm)	624.852	732.394	684.854
90 % D(µm)	762.522	874.687	787.442

The median particle size of N-acetyl-D-glucosamine is 44.67 μm (**Fig. 7** and **Table 4**). The particles range from 16.06 (10 %) to 391.76 (90 %) μm. The obvious discrepancy between sieve analysis and laser diffraction results can be explained by the electrostatic interaction of the substance with metal sieves which is attributable to the presence of an N-acetyl moiety. Apparently, this led to the remaining finer particles on the coarser mesh sieves. As a result, incorrect fractioning was observed.

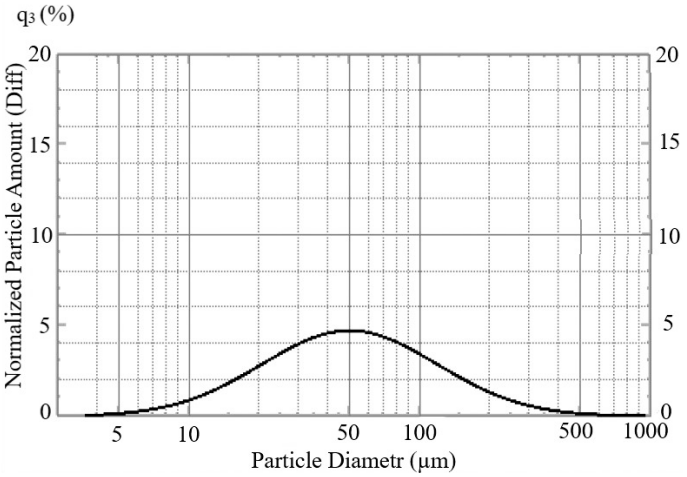


Fig. 7. N-acetyl-D-glucosamine particle size

Table 4
N-acetyl-D-glucosamine particle size distribution

Vol. Under	1st measurement	2nd measurement	3rd measurement
10 % D(μm)	16.065	16.005	16.063
20 % D(μm)	23.540	23.533	23.472
30 % D(μm)	31.207	31.294	31.053
40 % D(μm)	39.592	39.802	39.336
50 % D(μm)	49.427	49.796	49.043
60 % D(μm)	61.737	62.325	61.180
70 % D(μm)	78.295	79.203	77.491
80 % D(μm)	210.335	215.607	206.122
90 % D(μm)	382.883	391.756	375.931

Taking into account the median particle size of APIs, it is supposed that the mixture of them cannot be homogeneous. To verify it laser diffraction study of the mixture (Actimask® with N-acetyl-D-glucosamine in a 4:1 ratio) was performed directly after mixing and after exposure to vibration (simulating possible vibration and stratification during production). After vibration, samples were taken from three layers: upper, middle, and lower. The results were obtained by laser diffraction are shown in **Fig. 8** and **Table 5**.

The mixture before vibration shows a medium particle size of 446.68 μm which is greater than that of N-acetyl-D-glucosamine and corresponds to the Actimask®. But the fractional percentage has a larger range – from 194.43 (10 %) to 977.19 μm (90 %). This is due to the presence of N-acetyl-D-glucosamine, which is a fine powder. An increase in maximum size is possible due to the agglomeration of APIs. As it can be seen from **Fig. 8**, the upper and lower layers after vibration have similar particle size ranges – from 43.29 (10 %) to 506.25 (90 %) μm (for the upper layer) and 50.33 (10 %) to 508.92 (90 %) μm (for the lower layer). The mean particle size is 141.25 and 177.83 μm, respectively. At the same time, the middle layer of the mixture showed the mean particle size of 354.81 μm after vibration and particle size range from 142.99 (10 %) to 939.53 (90 %) μm.

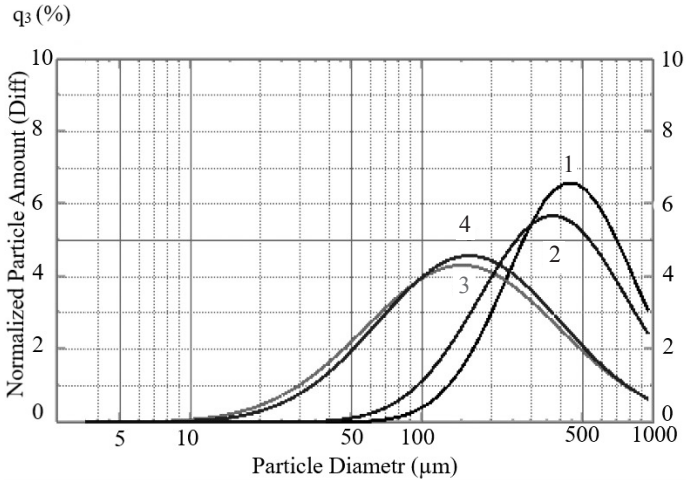


Fig. 8. Actimask® and N-acetyl-D-glucosamine mixture in 4 variants (1 – before vibration; 2 – after vibration middle layer sample; 3 – after vibration top layer sample; 4 – after vibration bottom layer sample)

Table 5

Particle size distribution of Actimask® Acetaminophen and N-acetyl-D-glucosamine mixture (4:1)

Vol. Under	1st measurement	2nd measurement	3rd measurement	4th measurement
10 % D(μm)	194.433	142.999	43.292	50.333
20 % D(μm)	256.637	199.036	66.213	74.510
30 % D(μm)	313.636	250.641	89.671	99.653
40 % D(μm)	370.963	304.766	116.409	127.240
50 % D(μm)	436.007	366.822	148.525	159.940
60 % D(μm)	511.407	442.104	189.442	201.023
70 % D(μm)	606.940	539.381	245.492	256.974
80 % D(μm)	743.542	680.840	333.423	343.509
90 % D(μm)	977.194	939.534	506.249	508.921

Thus, according to the results obtained, it may be supposed the heterogeneity of the mixture. The upper and lower layers have more fine particles, i.e. N-acetyl-D-glucosamine particles. The middle layer has more agglomerated particles extending beyond 1000 μm. N-acetyl-D-glucosamine sticks to Actimask® to form larger particles than pure substances. However, the adhesive properties of the substances appear to be insufficient for proper adhesion to each other.

In parallel, a chromatographic assay of APIs in tablets compressed from the mixture sampled before vibration and three layers formed after vibration was performed. The amount of acetaminophen and N-acetyl-D-glucosamine was determined experimentally in terms of 100 % of active substances. The results of chromatographic assay study are given in **Table 6**.

Table 6

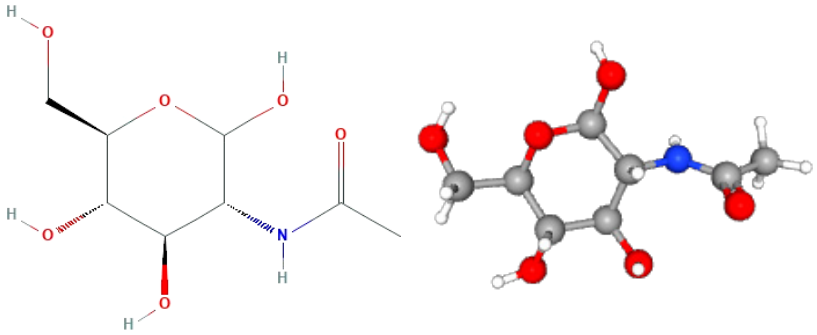
Results of chromatographic assay of APIs in tablets compressed from the mixture sampled before vibration and three layers formed after vibration

API	X_1, before vibration	X_2, upper layer	X_3, middle layer	X_4, bottom layer
$m_{paracetamol}$, mg (% of nominal weight)	396.70 (99.18 %)	431.05 (107.76 %)	412.73 (103.18 %)	435.53 (108.88 %)
$m_{glucosamine}$, mg (% of nominal weight)	103.70 (103.70 %)	68.95 (68.95 %)	87.27 (87.27 %)	64.47 (64.47 %)

The obtained data confirm the laser diffraction results on the occurrence of segregation in the API mixture. According to the requirements of European law [20] regarding the assay limits in medicines, the maximum acceptable deviation in the active substance content of the finished product shall

not exceed ± 5 % at the time of manufacture. That is, before vibration, the mixture met the abovementioned requirements, whereas exposing it to vibration has changed the quantitative ratio of APIs and the amount of Acetaminophen increased in all three layers. The lower and upper layers lost 35.53 and 31.05 % of N-acetyl-D-glucosamine, respectively, from its nominal content in tablets. The middle layer lost 12.73 % of N-acetyl-D-glucosamine, which, nevertheless, is also outside the acceptable limits.

It could be clearly seen that the percentage of N-acetyl-D-glucosamine did not increase in all three layers formed after vibration, but only reduced: to a greater extent in the upper and lower layer dosage units and a smaller extent – in the middle layer ones. Obviously, this fact could not be explained by the only mixture segregation in the tableting process. So, taking into account our previous results obtained in this work, especially those of sieve analysis and laser diffraction study, it is possible to conclude that inhomogeneity also occurs due to the sticking of N-acetyl-D-glucosamine to the metal vibrating cone. Sticking can be explained by electrostatic charging of glucosamine powder during friction against the funnel wall, most likely, due to amino group moiety (**Fig. 9**). Glucosamine has electrostatic properties that were demonstrated in the work of Yang Zhong et al. The paper describes the electrostatic activity of glucosamine particles between various substances in the liquid and gas phases [21].



a *b*

Fig. 9. Structural formula of N-acetyl-D-glucosamine: *a* – in 2D; *b* – in 3D

Thus, it could be assumed that passing the mixture being studied through a funnel during vibration cause adhesion of N-acetyl-D-glucosamine to the metal and, accordingly, reduction its content in all three mixture layers after vibration. A possible solution to the segregation problem, as well as electrostatic adhesion of powder to metal surfaces, is coarsening N-acetyl-D-glucosamine particles up to the size of Actimask® by means of granulation. The proposed approach is expected to reduce the electrification of N-acetyl-D-glucosamine.

4. Discussion

The results of our work convincingly evidence that the mixture of APIs with different particle sizes become segregated in the manufacturing process of the tablets. Although sticking of N-acetyl D-glucosamine particles to Actimask® (as supposed due to electrostatic activity of the first ones) was observed, it appeared to be not sufficient to prevent segregation. It should be noted that the conclusion was made based on several methods used in this study. Thus, the laser diffraction technique showed particle size distribution which was discrepant from sieve fraction analysis results that are supposed to be linked to electrostatic properties of N-acetyl D-glucosamine. Anyway, laser diffraction was considered as preliminary data which have to be verified by assay methods. HPLC study of the mixture after vibration exposure strongly indicated that segregation of the API powders occurs, so our further work will be aimed at searching for an optimal way to prevent segregation of the mixture.

The problem of powder segregation is an important technological aspect in the development of solid dosage forms, which has been described in earlier scientific works as well. E.g., M. Jaklič et al. have conducted an experimental study inspecting the segregation of direct compression mixtures of fillers and API with different particle sizes. For this purpose, straight pipes with different bottoms – hermetic and non-hermetic – were used. Segregation was detected by fluidizing the mixture: large particles are moved downwards while small particles are displaced upwards and attached to the

surface of the device [18]. In another work, the authors applied the simulation of fluidization followed by sampling and HPLC analysis of the mixture to determine whether it underwent segregation. It was found that segregation possibility is associated not only with different sizes of the component particles, but also the flow rate of the mixture during its pouring out [11]. L. Devriendt et al. have found that segregation, although dependent on the size of individual particles, is also affected by the amounts and ratio of the components. That is, it is possible to eliminate segregation by selecting the «ideal» ratio between large and small particles [22]. However, this way is applicable only in the case of API-excipient mixture, when the proportion of the latest may be varied.

Study limitations. Nevertheless, considering the findings of this study some limitations should be taken into account. In our opinion, the main limitation consists in the electrification of N-acetyl D-glucosamine particles as a result of the friction with metal parts of the tools. Other equipment that does not provoke electrification may be used to remove this restriction. However, on an industrial scale, metal equipment is commonly used, so in fact, this limitation reflects real manufacturing conditions. Another issue is that only vibration but not fluidization was applied to investigate segregation. However, the vibration as a way to cause segregation was chosen to take into account the smaller ratio of fine particles in the mixture and such a case sieving mechanism of segregation driven by vibration is most likely to occur.

The prospects for further research are to find a formulation approach allowing to obtain the homogenous mixture that will not be segregated in the process of tablet manufacture. A possible solution is to enlarge N-acetyl-D-glucosamine particles up to Actimask® Acetaminophen by the granulation technique.

5. Conclusions

1. Microscopic analysis was used to describe the morphology of Actimask® Acetaminophen and N-acetyl-D-glucosamine particles and their physical interaction with each other (sticking to each other). Surface roughness that can affect the pharmaceutical technical properties of the APIs and their mixture is revealed.

2. The pharmaceutical technical properties of APIs and their mixture, moisture absorption capacity, and the percentage of the particle size fractions are established. It is found that Actimask® has excellent flowability, while N-acetyl-D-glucosamine, on the contrary, is characterized by very poor flow. However, mixing these components results in the averaged values of the indicators. Acetaminophen and N-acetyl-D-glucosamine can be regarded as moisture-stable substances, but having particles of different sizes, which is a factor of possible segregation of their mixture.

3. The homogeneity of the mixture was checked by laser diffraction and chromatographic examination. A vibration funnel was used to simulate the effect of vibration during the production process. Segregation of the mixture under the effect of vibration, which can occur in production, was confirmed. The reason for the stratification of the mixture is probably the difference in particle size between the APIs and the physical properties of N-acetyl-D-glucosamine (a fine powder is prone to the formation of an electrostatic charge and sticking to the metal surfaces of the manufacturing equipment).

4. A possible solution to the problem of segregation of the mixture is coarsening the N-acetyl-D-glucosamine particles up to the size of Actimask® through granulation. The proposed approach is also expected to reduce the electrostatic activity of N-acetyl-D-glucosamine powder.

Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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References

[1] Deutsche Gesellschaft für Allgemeinmedizin und Familienmedizin. (2018). Chronischer Schmerz (AWMF-Registernr. 053/036). Available at: https://www.degam.de/files/Inhalte/Leitlinien-Inhalte/Dokumente/DEGAM-S1-Handlungsempfehlung/053-036%20chronischer%20Schmerz/S1-HE_Chronischer%20Schmerz_Langfassung.pdf

[2] Singh, M. K. (2021). Chronic Pain Syndrome Medication. Medscape. Available at: https://emedicine.medscape.com/article/310834-medication#4
[3] Chronic pain: supporting safer prescribing of analgesics (2017). British Medical Association. Available at: https://www.bma.org.uk/media/2100/analgesics-chronic-pain.pdf
[4] Nikles, C. J., Yelland, M., Del Mar, C., Wilkinson, D. (2005). The Role of Paracetamol in Chronic Pain: An Evidence-Based Approach. American Journal of Therapeutics, 12 (1), 80–91. doi: http://doi.org/10.1097/00045391-200501000-00011
[5] Rachin, A. P., Sharov, M. N., Averchenkova, A. A., Vygovskaya, S. N., Nuvakhova, M. B. (2017). Chronic pain: from pathogenesis to innovative treatment. RMJ, 9, 625–631. Available at: https://www.rmj.ru/articles/nevrologiya/Hronicheskaya_boly_ot_patogeneza_k_innovacionnomu_lecheniyu/
[6] Saleem, T. H., Abo El-Maali, N., Hassan, M. H., Mohamed, N. A., Mostafa, N. A. M., Abdel-Kahaar, E., Tammam, A. S. (2018). Comparative Protective Effects of N-Acetylcysteine, N-Acetyl Methionine, and N-Acetyl Glucosamine against Paracetamol and Phenacetin Therapeutic Doses–Induced Hepatotoxicity in Rats. International Journal of Hepatology, 2018, 1–8. doi: http://doi.org/10.1155/2018/7603437
[7] Reducing Powder Segregation – a big issue especially in vacuum conveying systems. LFA Machines. Available at: https://www.lfatabletpresses.com/articles/reducing-powder-segregation
[8] Understanding And Solving Segregation Effects In Pharmaceutical Solid-Dosage Form Operations. (2018). Pharmaceutical Online. Available at: https://www.pharmaceuticalonline.com/doc/understanding-and-solving-segregation-effects-in-pharmaceutical-solid-dosage-form-operations-0001#:%7E:text = Segregation%20of%20powder%20and%20granular,in%20an%20inconsistent%20final%20product
[9] Particle Segregation Testing Instrument (2018). Pharmaceutical Online. Available at: https://www.pharmaceuticalonline.com/doc/spectester-particle-segregation-tester-0001
[10] Delft Solids Solutions. (2016). Segregation of Powders (No. 2). Available at: https://www.solids-solutions.com/wp-content/uploads/2016/05/Segregation.pdf
[11] Shah, K. R., Farag Badawy, S. I., Szemraj, M. M., Gray, D. B., Hussain, M. A. (2007). Assessment of Segregation Potential of Powder Blends. Pharmaceutical Development and Technology, 12 (5), 457–462. doi: http://doi.org/10.1080/10837450701556834
[12] Skibsted, E. T. S., Boelens, H. F. M., Westerhuis, J. A., Witte, D. T., Smilde, A. K. (2006). Simple assessment of homogeneity in pharmaceutical mixing processes using a near-infrared reflectance probe and control charts. Journal of Pharmaceutical and Biomedical Analysis, 41 (1), 26–35. doi: http://doi.org/10.1016/j.jpba.2005.10.009
[13] Chung, Y.-C., Liao, C.-C., Zhuang, Z.-H. (2021). Experimental investigations for the effect of fine powders on size-induced segregation in binary granular mixtures. Powder Technology, 387, 270–276. doi: http://doi.org/10.1016/j.powtec.2021.04.034
[14] Fan, Y., Hill, K. M. (2015). Shear-induced segregation of particles by material density. Physical Review E, 92 (2). doi: http://doi.org/10.1103/physreve.92.022211
[15] Arntz, M. M. H. D., Beeftink, H. H., den Otter, W. K., Briels, W. J., Boom, R. M. (2013). Segregation of granular particles by mass, radius, and density in a horizontal rotating drum. AIChE Journal, 60 (1), 50–59. doi: http://doi.org/10.1002/aic.14241
[16] Liao, C.-C., Ou, S.-F., Chen, S.-L., Chen, Y.-R. (2020). Influences of fine powder on dynamic properties and density segregation in a rotating drum. Advanced Powder Technology, 31 (4), 1702–1707. doi: http://doi.org/10.1016/j.apt.2020.02.006
[17] Linek, J. (2007). Annual Report 2006. Institute of Chemical Process Fundamentals of the ASCR, V.V.I. Prague.
[18] Jaklič, M., Kočevar, K., Srčič, S., Dreu, R. (2015). Particle size-based segregation of pharmaceutical powders in a vertical chute with a closed bottom: An experimental evaluation. Powder Technology, 278, 171–180. doi: http://doi.org/10.1016/j.powtec.2015.03.021
[19] State Pharmacopoeia of Ukraine (SPU). Vol. 1 (2015). SE «Pharmacopoeia Centre.»
[20] Commission Directive 2003/63/EC (2003). The Official Journal of the European Union, L159.
[21] Zhong, Y., Bauer, B. A., Patel, S. (2011). Solvation properties of N-acetyl-β-glucosamine: Molecular dynamics study incorporating electrostatic polarization. Journal of Computational Chemistry, 32 (16), 3339–3353. doi: http://doi.org/10.1002/jcc.21873
[22] Devriendt, L., Gatumel, C., Berthiaux, H. (2013). Experimental Evidence of Mixture Segregation by Particle Size Distribution. Particulate Science and Technology, 31 (6), 653–657. doi: http://doi.org/10.1080/02726351.2013.832447

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