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DEVELOPMENT OF NEW FORMULATION OF INOSINE ACEDOBEN DIMEPRANOL POWDER FOR ORAL SOLUTION IN SACHETS

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Abstract. *Inosine Acedoben Dimepranol (IAD) has immunomodulatory and antiviral properties and is used in the treatment of viral infections. The aim of the present study was to develop a powder for oral solution in sachets containing high dose IAD (1000 mg). The formulations were prepared by direct dry mixing of the components using the dilution principle. Optimal composition and process parameters for the development of high dosage powder were established. Solubility studies showed pH-dependent solubility of IAD. The prepared powder was characterized by a short reconstitution time. In addition, the oral solution exhibits good organoleptic properties and remains stable for 30 min. Stability studies indicated that the sachets remained stable under accelerated conditions for 3 months. In conclusion, the developed powder for oral solution in sachets containing 1000 mg IAD represents an economical alternative to other solid dosage forms with the advantages of rapid dissolution and convenient intake for the patient.*

Key words: *Inosine Acedoben Dimepranol (IAD), powder for oral solution in sachets, reconstitution, solubility, spray-dried mannitol, stability*

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INTRODUCTION

Inosine Acedoben Dimepranol (IAD) also known as Inosine pranobex and Methisoprinol is a synthetic purine derivative with immunomodulatory and antiviral properties. It has been widely used in the treatment of viral infections caused by different herpes viruses, human papilloma viruses, human immunodeficiency virus as well as viral diseases such as herpes zoster, varicella, measles, subacute sclerosis panencephalitis, influenza, acute respiratory infections and others. IAD has been proven to positively impact the host's immune system by enhancing T-cell lymphocyte proliferation and activity of natural killer cells, increasing levels of proinflammatory cytokines, and thereby restoring deficient responses in immunosuppressed patients. At the same time, it has been shown to affect viral RNA levels and hence inhibit growth of several viruses [1, 2]. Due to its potent immunomodulatory properties as well as its safety profile IAD has been intensively evaluated for its potential as COVID-19 medication [3]. Most often, including in COVID-19 inosine pranobex is administered in dose of 1000 mg three to four times daily in adults (maximum 4 g day^{-1}) [4].

The drug was developed by Newport Pharmaceuticals in the 1970s and distributed in more than 80 countries under the trademarks Isoprinosine® and Imunovir® (owned by Newport) for the treatment of

viral infections. The active substance is a compound (complex) of inosine (1,9-dihydro-9-Dribofurasonyl-6H-purin-6-one) and a salt of 4-acetamidobenzoic acid with N, N dimethylamino-2-propanol in a molar ratio of 1:3. It is a crystalline powder with a white to cream color and a characteristic odor, freely soluble in water, sparingly soluble in methanol, acetone and ethanol [5, 6]. It is interesting that the active ingredient is not included in any pharmacopoeia.

In connection with recent prescriptions of a high single dose of 1000 mg, there is a need to develop a high-dosage form of IAD such as a powder for oral solution in a sachet. Furthermore, polypharmacy, dysphagia and age-related pathological changes in older adults present challenges for medication management. In addition, as many as one in five older patients has difficulty swallowing and may have problems taking tablets and capsules [7].

In the pharmaceutical and nutraceutical industries sachets are used for many types of single-dose, dry powder formulations – from pain medications, products for cold and flu treatment to vitamins, mineral mixes and probiotic preparations. Granules for oral solution in a sachet containing 1000 mg of IAD have recently been available on the market in European countries [8]. Granules are premade agglomerates of smaller particles of powder. They are prepared by wet and dry methods. However, the preparation of the granules involves several processing steps and takes more time, energy, and space, hence, it is a costly technique. It is difficult to formulate active pharmaceutical ingredients, which are hygroscopic or deliquescent, amorphous, oxygen-sensitive and/or volatile. The dosage form “powder for oral solution in sachet” of IAD (1000 mg) allows rapid dissolution of the active substance and is a convenient alternative to tablets for some patients such as those with dysphagia. In addition, sachets offer excellent dose accuracy. One sachet is dissolved in water immediately before oral administration. Furthermore, manufacturing of powder dosage form is a simple and economic process, hence, the product cost is lower as compared to other dosage forms. Dissolution rate of oral powders containing water-soluble drugs is generally faster than granules, tablets or capsules, in which disintegration of the tablet or the capsule shell is required prior to dissolution.

Currently, a product containing 1000 mg inosine pranobex in the form of powder for oral solution is available in Bulgaria. However, the aim of the present work was to develop a stable drug product in the form of powder for oral solution containing a high dose of inosine pranobex (1000 mg) with a different composition in order to achieve better dosage form properties. An additional objective was to compare the impact of different types of mannitol on the characteristics of the final product.

MATERIALS AND METHODS

The following materials were used for the purpose of the present study: Inosine Acedoben Dimepranol (ABC Farmaceutici S.p.A., Italy), mannitol (Pearlitol® 200 SD, Roquette; Mannogem® EZ SD, SPI), Powder strawberry flavor (Robertet), Acesulfame potassium (Anhui Jinhe Industrial Co., Ltd), Aspartame (Ajinomoto Sweeteners Europe SAS), Silica Colloidal Anhydrous (Aerosil® 200, Evonik); all excipients meet the requirements of the European Pharmacopoeia (Ph. Eur.).

Preparation of powder for oral solution

One series of laboratory batches were prepared in the form of direct powder mixtures in order to establish the optimal content of the diluent, mannitol (Mannogem® EZ SD). The size of the prepared lab batches was 100 sachets. All ingredients were accurately weighed and sieved through a 0.80 mm sieve. The product was prepared in the form of direct powder mixture by a process of mixing the components in a diffusion mixer (diffusion). First, a premix of IAD, half the amount of mannitol and silica colloidal anhydrous is prepared (mixing time 15 min at a rate of 25 rpm). A final mixture is obtained by adding the remaining ingredients to the mixer and homogenizing for additional 20 min at the same rate. The mixture is dosed in sachets (Paper/AL/PE) according to the determined unit mass for the composition.

In addition to the abovementioned powder mixtures, a powder (IAD L04) containing different diluent (Pearlitol® 200 SD instead of Mannogem® EZ SD) was also prepared. The process of mixture development is the same as described above. The compositions of the batches (IAD L01 – IAD L04) are presented in Table 1.

Characteristics of powder mixtures

Characteristics of the finished powders

Flow properties of the powder mixtures were evaluated using the Hausner ratio according to Ph. Eur. Bulk density and tapped density measurements were performed according to the method of Ph. Eur. [9, 10].

Average mass of contents of a single sachet

Measurements were performed according to the method of Ph. Eur. (European Pharmacopoeia 10th ed. 2019). The outer surface of the package was clean. The package was opened and measured immediately together with the contents. The contents

Table 1. The compositions of lab batches

Ingredients	Batch No								
	IAD L01 mg/sachet	% w/w	IAD L02 mg/sachet	% w/w	IAD L03 mg/sachet	% w/w	IAD L04 mg/sachet	% w/w	IAD L04 g/200 sachets
Inosine Acedoben Dimepranol	1000.00	25.38	1000.00	27.78	1000.00	29.41	1000.00	29.41	200.00
Mannitol (Mannogem® EZ SD or Pearlitol® 200 SD)	2863.00	72.66	2523.00	70.08	2323.00	68.32	2323.00	68.32	464.60
Strawberry flavor (powder)	54.00	1.37	54.00	1.50	54.00	1.59	54.00	1.59	10.80
Acesulfame potassium	10.00	0.25	10.00	0.28	10.00	0.29	10.00	0.29	2.00
Aspartame	8.00	0.20	8.00	0.22	8.00	0.24	8.00	0.24	1.60
Silica colloidal anhydrous (Aerosil® 200)	5.00	0.13	5.00	0.14	5.00	0.15	5.00	0.15	1.00
Total mass	3940.00	100.00	3600.00	100.00	3400.00	100.00	3400.00	100.00	680.00

were evacuated as much as possible in full. The weight of the contents was calculated as the difference between the two measurements. The procedure was repeated with nine packs.

Solubility studies of IAD

According to data of the manufacturer, inosine pranobex is described as freely soluble in water but the biopharmaceutics classification system (BCS) requires data on the solubility at 37 ºC and in different media with different pH values. For that reason, the following solubility tests were undertaken: to different samples of IAD (approximately 10.0 mg, 50.0 mg and 690.0 mg) in a glass-stoppered graduated cylinder (10.0 mL) were added increasing volumes of solvent at 20 ºC and 37 ºC. After each addition of the solvent to the indicated total volume, the mixture was shaken vigorously for 10 min and was visually checked for any undissolved parts in the sample.

Scanning electron microscopy (SEM)

Photos of the diluent Pearlitol® 200 SD, active substance IAD and a mixture of them in a ratio of 1:1 were obtained by Analytical scanning electron microscope JSM-6010PLUS/LA (JEOL Company).

Stability studies

Tests for the stability of the product samples under accelerated conditions (40 ºC / 75% RH) for 3 months were performed according to the requirements of the guidance on stability testing: Stability testing of new drug substances and products [10, 11].

Reconstitution time

The contents of one sachet were transferred to a beaker containing 100.0 mL of water, maintained at 20 ± 2° C. The mixture was stirred for 5 min as the temperature being maintained within the limits shown. The procedure was repeated for another 5 sachets. The test is valid if all six sachets to dissolve for 5 min. A clear to opalescent solution should be obtained.

pH of the oral solution

The potentiometric determination of *pH* was made by *measuring* the potential difference between two appropriate electrodes immersed in the prepared oral *solution [9].*

High-performance liquid chromatographic (HPLC) analysis

HPLC analysis of inosine pranobex in the developed powders

A high-performance liquid chromatographic (HPLC) method was developed for quantitative determination of IAD in the medicinal product [5, 12]. For the HPLC analysis was used a chromatograph with a variable wavelength detector. The analysis was performed on an analytical column packed with octadecylsilyl silica gel (RP-18) (150 mm x 4.6 mm, 5.0 µm). Two mobile phases were used and both were a mixture of double-distilled water and methanol in different ratios. Mobile phases were filtered through a Millipore 0.45 µm filter and degassed. Systemic control, data collection and analysis were performed using the chromatography software. Chromatographic conditions are presented on Table 2.

For the test solution preparation, approximately 0.7 g of the powder were measured corresponding to 200.0 mg IAD. Then, 40.0 to 50.0 mL of water were added and homogenized. After that the solution was diluted to 100.0 mL with water and treated in an ultrasonic bath for 5 min. The sample was cooled and filtered through a membrane filter with a pore size

Table 2. Chromatographic conditions/Gradient mode and conditions

Flow rate		**2.0 mL/min**	
Column temperature		30° C	
Analytical wavelength		254 nm	
Injection volume		20.0 mL	
Solvent		A mixture of water and methanol (90:10%, v/v)	
Duration of chromatographic analysis		8.5 min	
Gradient mode and conditions			
Time (min)	Mobile phase A: water/methanol (80:20%, v/v) containing 0.1 % phosphoric acid	Mobile phase B: water/methanol (60:40%, v/v)	
2.0	100	0	isocratic
0.5	0	100	linear gradient
3.5	0	100	isocratic
0.5	100	0	linear gradient
2.0	100	0	isocratic

as 2.65 g of dipotassium hydrogen phosphate were dissolved to 1000.0 mL with water. The pH of the solution was adjusted to 2.5 with orthophosphoric acid (80 %). Chromatographic conditions of the analysis are shown on Table 3.

Table 3. Chromatographic conditions

Flow rate		**1.0 mL/min**	
Column temperature		25° C	
Analytical wavelength		254 nm	
Injection volume		20.0 mL	
Duration of chromatographic analysis		40 min	
Gradient mode and conditions			
Time (min)	Mobile phase A: buffer (pH 2.5)/ methanol (95:5%, v/v)	Mobile phase B: buffer (pH 2.5)/methanol (50:50%, v/v)	
10	90	10	isocratic
5	80	20	linear gradient
15	80	20	isocratic
10	90	10	linear gradient

of 0.45 mm (suitable Nylon). Subsequently, 1.0 mL of the filtrate was diluted to 50.0 mL with water. A reference solution was prepared as 20.0 mg of IAD (standard substance) were dissolved in water and diluted to 50.0 mL with the same solvent. 1.0 mL of the resulting solution was diluted to 10.0 mL with water. Then, the suitability of the chromatographic system was tested. For the purpose, the column was conditioned for 30 min, then 20.0 µL of the reference solution were introduced in sixfold. The retention times are about 1.2 min for inosine pranobex and about 5.5 min for 4-acetamidobenzoic acid salt. The total content (X) in mg per sachet was calculated by the formula:

$$X = 0.2405*Xi + 0.7595*X_{4\text{-acet}}$$

where:

X_i = IAD content, such as inosine (mg);

$X_{4\text{-acet}}$ = IAD content, such as 4-acetamido benzoic acid salt (mg)

HPLC analysis of related substances

A HPLC method was developed for quantitative determination of the following impurities: hypoxanthine and 4-aminobenzoic acid [6]. The mobile phases used were mixtures of buffer (pH 2.5) and methanol in different ratios. The buffer (pH 2.5) was prepared

Test solution was prepared as 0.17 g of the powder corresponding to 50.0 mg IAD were accurately weighed and dissolved in water and then diluted to 50.0 mL with water. The obtained solution was filtered through a microporous filter with a pore size of 0.45 µm. The following reference solutions were prepared for the analysis:

- Reference solution (1): 20.0 mg of 4-aminobenzoic acid was dissolved in 5.0 mL of methanol and diluted to 50.0 mL with water.
- Reference solution (2): 20.0 mg of hypoxanthine was dissolved in 5.0 mL of methanol and diluted to 50.0 mL with water.
- Reference solution (3): 1.0 mL of the reference solution (1) and 4.0 mL of the reference solution (2) was placed in a volumetric flask (10.0 mL) and diluted to 10.0 mL with water.
- Reference solution (4): 50.0 mg of IAD (standard substance) was dissolved in 20.0 mL of water. Then, 1.0 ml of reference solution (3) was added and the obtained solution was diluted to 50.0 mL with water and homogenized.
- Reference solution (5): 1.0 mL of each reference solution (1 and 2) was placed in a 20.0 mL volumetric flask and diluted to volume with water. After

that, 1.0 mL of the resulting solution was diluted to 10.0 mL with water.

- Reference solution (6): 1.0 mL of the test solution was diluted to 50.0 mL with water. After homogenizing, 1.0 mL of the resulting solution was diluted to 10.0 mL with water.
- The relative retention times were: about 0.74 min for hypoxanthine, about 3.9 min for inosine pranobex, about 2.1 min for 4-amino benzoic acid and about 8 min for 4-acetamido benzoic acid salt.

HPLC analysis of the resulting oral solution after reconstitution of one sachet in water

The content of one sachet was dissolved in 100.0 mL of drinking water with temperature 20 ºC under stirring. The time for obtaining clear solution was determined. At determinate intervals the solution was analyzed for the content of active substance and its impurities. The method for this analysis was the same as described above.

RESULTS

Preparation of powder for oral solution

The most commonly used diluents in the dosage form powder for oral solution are water-soluble sugars and polyols such as mannitol or sugar [13]. The composition of the powder mixture was chosen considering physical properties of IAD and some excipients (Table 4).

According to the results, grades of mannitol have the most appropriate bulk density, which has a similar value to that of the active substance inosine pranobex.

Characteristics of powder mixtures

Solubility studies of IAD

According to the Guideline on the investigation of bioequivalence, the active substance is considered to be very soluble if the highest dose administered as an immediate release form is completely dissolved in 250.0 mL of buffers with a pH range of 1 to 6.8 at 37 ± 1 °C [13]. The results of the solubility studies of IAD in different conditions are presented in Table 5 and Fig. 1.

Effect of different grades of mannitol

According to the listed physical characteristics in Table 4, little differences are seen between the two spray-dried grades of mannitol (Mannogem® EZ SD and Pearlitol® 200 SD), so that they may be interchangeable. Thus, batch No. IAD L04 was laboratorily prepared with the optimal composition of batch No. IAD L03, but Pearlitol® 200 SD was used instead of Mannogem® EZ SD. The composition of batch No.

Table 4. Physical characteristics of IAD and some excipients

Ingredient	Particle Size (μm)	Bulk density (g/ ml)	Tapped Density (g/ ml)	Criterion of Hausner (< 1.25)
Inosine Acedoben Dimepranol	100 %, < 300	0.370	0.526	1.42
Compressible sugar (DI-PAC)	75 %, 150	0.667	0.833	1.25
Mannitol (Mannogem® EZ SD), SPI	75 ÷150	0.500	0.556	1.11
Mannitol (Pearlitol® 200 SD), Roquette	d50 = 180	0.513	0.588	1.15

Table 5. Solubility of IAD in different conditions

Solvent-media, temperature	Approximate solubility of IAD (mg/mL)	Approximate solubility of IAD (%, w/v)	Criterion: Dose 1000 mg (D) : Solubility (S) ratio, D:S < 250.0 mL (37º C)
Purified water, 20º C	230.0	23.0	
Purified water, 37º C	345.0	34.5	2.17
HCL, 0.1 mol/l, pH = 1.2, 20º C	< 1.0	< 0.1	
HCL, 0.1 mol/l, pH = 1.2, 37º C	< 1.0	< 0.1	> 250.0
Buffer, pH = 4.5, 20º C	153.3	15.3	
Buffer, pH = 4.5, 37º C	345.0	34.5	2.17
Buffer, pH = 6.8, 20º C	230.0	23.0	
Buffer, pH = 6.8, 37º C	345.0	34.5	2.17

IAD L03 was chosen due to the lowest mannitol content. The size of the batch is sufficient for its investigation and analysis.

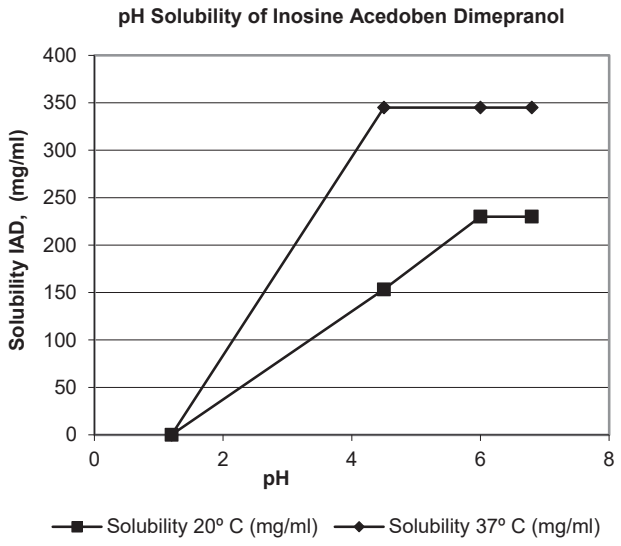


Fig. 1. pH-dependent solubility of IAD

The results obtained confirmed that Pearlitol® 200 SD imparts improved flow properties to the formulation in the same degree as Mannogem® EZ SD. There are no differences also in the other characteristics such as reconstitution time, appearance of the solution and organoleptic properties. The results of the tested characteristics of the experimental batches (IAD L01 – IAD L04) are presented in Table 6.

According to the Hausner ratio, flowability of the active substance inosine pranobex is in the range of values that indicate bad flow character (HF = 1.42). However, the data for the Hausner ratio of the tested batches product shown values < 1.25, i.e., all batches exhibit good flow properties.

Reconstitution

The results from the conducted tests showed comparative and short reconstitution times for all batches – up to 2.5 min. Furthermore, the formulations exhibit good organoleptic properties of the reconstituted solution. It has a pleasant taste without marked bitterness and with strawberry odor. Therefore, the diluent used is suitable for the developed powder formulation.

Blending time effect

Proper blending shall be established by checking content uniformity of the drug at all the time intervals studied. For the study, batch No. IAD L04 was taken. The measured response was content uniformity and RSD. The acceptance criteria were 100±15 % (RSD not more than 6.0%). Results are shown in Fig. 2.

The distribution of IAD is well acceptable according to the predetermined specification of all mixing intervals as shown by the analyzed samples. However, after 20 minutes a closer homogeneity of the distribution of IAD with the other excipients of the mixture was observed. In addition, the results of the 20-minute analysis have the lowest standard deviation value.

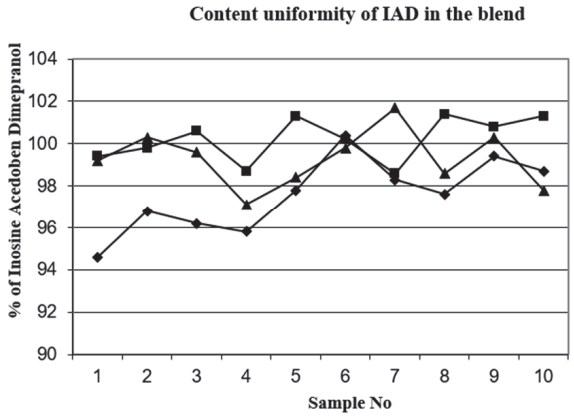
Scanning electron microscopy (SEM)

The spray-dried mannitol diluents are roughly spherical in shape and porous as it can be seen in Fig. 3A. Particle shape may significantly affect the extent of particle surface contact, and therefore, the magnitude of van der Waal's short-range forces [14, 15]. Scanning electron microscopic studies of the powder mixtures confirmed the assembly of fine particles on the diluent surface discontinuities during mixing (Fig. 3).

Table 6. Results from tested parameters for the first three batches

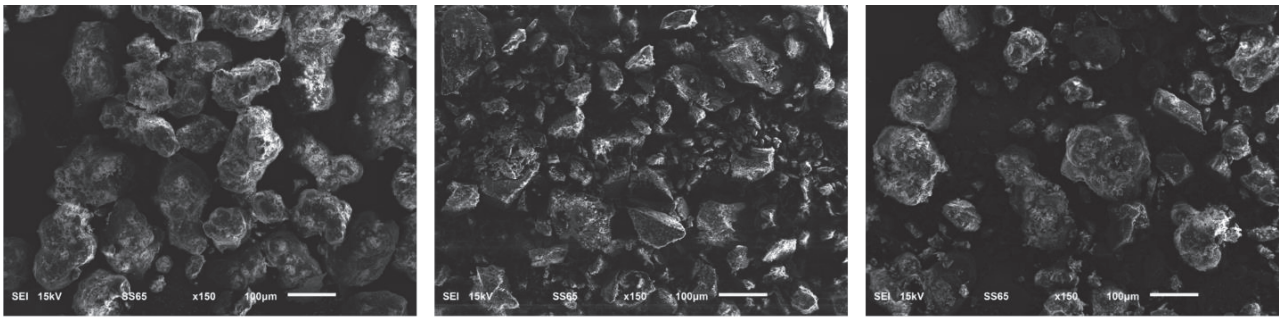
Tested parameters	Evaluation			
	Batch No. IAD L01	Batch No. IAD L02	Batch No. IAD L03	Batch No. IAD L04
Bulk density, ρo (g/mL)	0.477 ± 0.02	0.477 ± 0.02	0.500 ± 0.04	0.500 ± 0.04
Tapped density, ρs (g/mL)	0.557 ± 0.03	0.557 ± 0.03	0.583 ± 0.03	0.583 ± 0.03
Hausner ratio, HF = ρs/ρo	1.167 ± 0.01	1.167 ± 0.01	1.168 ± 0.03	1.168 ± 0.03
Reconstitution time (min)	2.5 ± 0.05	2.5 ± 0.05	2.5 ± 0.10	2.5 ± 0.10
Appearance of the solution	Clear, colorless solution	Clear, colorless solution	Clear, colorless solution	Clear, colorless, solution
pH of the solution	6.31 ± 0.02	6.42 ± 0.03	6.46 ± 0.03	6.28 ± 0.02
Organoleptic properties	Pleasant taste without marked bitterness, with strawberry odor	Pleasant taste without marked bitterness, with strawberry odor	Pleasant taste without marked bitterness, with odor	Pleasant taste without marked bitterness and with strawberry odor

All values are reported as mean ± SD (n = 3)



No Samples	% of Inosine Acedoben Dimepranol		
	15 min	20 min	25 min
1	94.6	99.4	99.2
2	96.8	99.8	100.3
3	96.2	100.6	99.6
4	95.8	98.7	97.1
5	97.8	101.3	98.4
6	100.4	100.2	99.8
7	98.3	98.6	101.7
8	97.6	101.4	98.6
9	99.4	100.8	100.3
10	98.7	101.3	97.8
Mean	**97.56**	**100.21**	**99.28**
STDEV	1.75	1.05	1.35
RSD %	**1.80**	**1.05**	**1.36**

Fig. 2. Content Uniformity results of IAD in the blend of Batch No. IAD L04



A: Scanning electron microscopy of spray-dried mannitol (Pearlitol® 200 SD)

B: Scanning electron microscopy of IAD

C: Scanning electron microscopy of Pearlitol® 200 SD blended with IAD

Fig. 3. Scanning electron microscopic photos

Stability studies

Batch No. IAD L04 powder for oral solution was packed in three-layer sachets (polyethylene foil – aluminum – paper, PE-Al-P) and stability tests under accelerated conditions (40ºC /75 % RH) for 3 months were conducted. The analytical results are presented in Table 7.

The results of the batch impurity profile after 3 months of storage under accelerated conditions did not show any significant changes. In addition, no changes in the physicochemical parameters of the sachets were observed. The results correspond to the product specification.

HPLC ANALYSIS OF THE RESULTING ORAL SOLUTION AFTER RECONSTITUTION OF ONE SACHET IN WATER (BATCH NO. IAD L04)

The oral solution prepared from one sachet (batch No. IAD1 L04) was analyzed for the quantitative content of the active ingredient and its impurities in certain intervals of time from the time of reconstitution. Furthermore, the test aimed to assess the duration of the reconstituted solution stability for oral administration. The sample was tested three times. Results are shown in Table 8.

The data showed that the quality of the reconstituted oral solution (IAD 1000 mg/sachet) corresponds to the declared content of the active substance (100 ± 5 %).

DISCUSSION

The development of a suitable formulation of IAD in the form of a single-dose sachet containing a free flowing powder for reconstitution is related to the determination of several basic problems: the choice of a suitable water-soluble diluent assuring simultaneously flow properties of the powder mixture, uniformly distribution of the active substance in the mixture, stability of the powder mixture and acceptable characterization of the process of dissolving, the stability of the solution and availability of the active substance up to administration, good organoleptic properties of the oral solution without any bitterness by taste and odor masking of the active substance [16].

Spray-dried mannitol was considered the most appropriate diluent for the powder composition consid-

Table 7. Stability studies of batch No. IAD L04 (40 ºC /75% RH) for 3 months

No	Name of parameter	Units	Characteristics and norms for batch release	Results
1.	Appearance	–	White or almost white powder with strawberry-like odor	Compliance
2.	Identification – UV absorption – HPLC	–	Compliance with the tests	 Positive Positive
3.	Average mass of content:	g/sachet	3.145 to 3.655	3.418
4.	Uniformity of dosage units, mass variation	%	AV ≤ 15	2.4
5.	Characteristic of oral solution			
5.1	Reconstitution time	min	Not more than 5	2.5
5.2	Appearance of oral solution	–	Clear to slightly opalescent	Clear
5.3	Odor of solution		Strawberry-like	Compliance
5.4	pH of oral solution	–	5.8 to 7.0	6.31
6.	Related substances (HPLC)	%		
6.1.	Hypoxanthine	%	not more than 0.2	0.04
6.2.	4-aminobenzoic acid	%	not more than 0.2	Undetectable
6.3.	Total impurities	%	not more than 0.5	0.04
7.	HPLC analysis Inosine (theoretical 24.05%) 4-acetamido benzoic acid salt (Theoretical 75.95%) Sum of the active components	mg/sachet	 240.50 (95.0-105.0 %) 759.50 (95.0-105.0 %) 1000.00 (95.0-105.0 %)	 245.2 773.5 1018.7

Table 8. Analysis of the solution produced at 20 ºC (batch No. IAD L04, 1000 mg IAD/sachet)

Time (min)	HPLC analysis, mg (%)			Related substances HPLC, %		
	Inosine	4-acetamido benzoic acid salt	Sum of active components	Hypoxanthine	4-amino-benzoic acid	Total impurities
0	241.4 (100.4%)	774.3 (101.9%)	1015.7 (101.6%)	ND	ND	ND
10	244.6 (101.7%)	776.2 (102.2%)	1020.8 (102.1%)	ND	ND	ND
20	244.3 (101.6%)	773.9 (101.9%)	1018.2 (101.8%)	ND	ND	ND
30	242.7 (100.9%)	774.9 (102.0%)	1017.6 (101.8%)	ND	ND	ND

Acronym: ND = not detected

ering the physical properties of IAD. The spray-dried grade of mannitol is characterized with high porosity and narrow particle size which provides strong adsorption sites for fine particles in the powder mixture and make it an ideal choice for triturating powders. Spray-dried mannitol has spherical particles giving good flowability and content uniformity. Narrow particle size distribution helps reduce segregation. Mannitol is non-hygroscopic in contrast to sucrose which is an important advantage in the manufacturing process. In addition, mannitol is suitable for administration in diabetic patients. Both grades of mannitol used in the present study have similar characteristics, with the exception that they have different mean particle size as well as different particle size distribution which may result in different flow properties of the final product [17].

IAD is acid insoluble and therefore not “highly soluble” as defined in the present BCS guidelines. IAD meets criterion D:S < 250.0 mL in medium with a pH between 4.5 and 6.8. By increasing the temperature from 20 ºC to 37 ºC, the solubility of IAD was increased by about 1.5 times. The dose of 1000 mg IAD will be dissolved readily in a glass of water before the administration.

Furthermore, even at 20º C only 5.0 mL of water will be sufficient for its complete dissolving.

Inosine pranobex is characterized with slight bitter taste and specific odor. Often the bitter taste of drug products leads to lack of patient compliance, especially in children. In order to create a palatable drug delivery system, other factors should be combined with the masking system. These include a sweetness profile, which is designed for the specific active substance and/or a system used to deliver the active substance, together with a flavor, which is formulated specifically for the system involved. A variety of high-intensity sweeteners that can be used either alone or in combination with each other to provide a specific sweetness profile are available. We have found that Acesulfame potassium, which has an initial burst of sweetness but dissipates rapidly used in combination with Aspartame, which has a tendency to build in intensity over time, produces a sweetness profile, which extends itself over the time, during which the product is experienced in the mouth. A strawberry flavor was created to complement the masking and sweetness profiles. The optimal content as well as the sweeteners ratio were achieved during the preliminary experiments.

After the establishment of a suitable leading formulation and control of the most critical parameter, further batches were made at laboratory scale (around 680 g) in order to investigate the robustness of the formulation with regard to variations in the mannitol grade and the blending conditions. As it was mentioned, according to the Hausner ratio of the tested batches, all batches exhibit good flow properties. In addition, the result showed that decreasing content of mannitol in the composition (from 72.66% to 68.32%) and respectively the mass of the sachet (from 3.94 g to 3.4 g) did not change the good flow properties of the powder mixtures. It is important to minimize the content of mannitol due to its laxative properties in large doses. Thus, the batch composition No. IAD L03 was determined as the composition with optimal content of the diluent (Mannogem® EZ SD) and was used for the preparation of batch No. IAD L04 where the diluent Mannogem® EZ SD was replaced by Pearlitol® 200 SD. Batch No. IAD L04 was subsequently analyzed in order to evaluate the influence of spray-dried grades of mannitol as well as the blending time on powder characteristics. The purpose of blending is to get a uniform distribution of the active substance. Mixing speed and time are critical variables in this process [14, 15]. Since the speed of the blender is constant (25 rpm), proper mixing time shall be determined. Mixing is critical as less blending will result in non-uniform distribution of the drug and poor flow, whereas more blending will result in de-mixing leading to non-uniform distribution of the drug and increase in the disintegration time.

The supposed mixing mechanism is “ordered mixing” because the diluent (spray-dried mannitol) ensures morphology necessary for the process– surface contributing to the retention of the particles of IAD via weak hydrogen bonds. The comparison of the measured value of the bulk density of the blend (Batch No. IAD L03) - ρo measured and the calculated value - ρo calculated was used for evaluation of the effect of “ordered mixing”. If ρo measured > ρo calculated that shows the presence of the effect of “ordered mixing” or making the blend more tighter.

The value ρo calculated was calculated according to the equation:

$$1/\rho_o \text{ calculated} = x1/\rho1 + x2/\rho2 + x3/\rho3 + \ldots x_n/\rho_n$$

where:

$x1, x2, x3 \ldots\ldots x_n$ are the percentage contents of the single ingredients of the blend

$\rho1, \rho2, \rho3 \ldots\ldots \rho_n$ – corresponding bulk densities of the ingredients.

1/ρo calculated = 0.294/0.370active + 0.683/0.500 mannitol + 0.023/0.500other = 0.79 + 1.36 + 0.05 = 2.2

ρo calculated = 1/2.2 = 0.454

ρo measured = 0.500

ρo measured > ρo calculated

Consequently, during the mixing of IAD and spray-dried mannitol the process of “ordered mixing” proceeds [18]. That mixture exhibits a high degree of homogeneity and content uniformity of IAD. The stability of an ordered mixture depends on the cohesive forces acting between the fine particles and the forces of adhesion of the fine particles to the coarse diluent particles. The adhesion forces should exceed the cohesive forces in order to obtain uniform and stable ordered mixtures. In the literature, ordered mixtures have also been referred in other terms, like “regimented” and “structured mixtures” [14, 15].

An essential characteristic of the dissolving process of the prepared powder in water is its rate or reconstitution time, i.e., the necessary time for the dissolving of the active substance and the whole formulation. All the batches are characterized short reconstitution time (approximately 2.5 min). Furthermore, similarly to the tablets, the product provides 100% of the active ingredient in the solution, but it can be absorbed and reach the bloodstream faster than the tablet forms. The solution remains stable for 30 minutes. In addition, the profile of impurities of IAD in solutions is far lower than the specified limits for 30 minutes:

hypoxanthine – not more than 0.2%; 4-aminobenzoic acid – not more than 0.2% and total impurities – not more than 0.5%. HPLC analysis of the resulting oral solution after reconstitution of one sachet in water showed that the determined quantity of IAD (1000 mg/sachet) corresponds to the declared content of the active substance (100 ± 5 %). These results confirm the appropriateness and optimality of the processes in the development of new dosage form for inosine pranobex.

CONCLUSIONS

According to the results of the study, an optimal composition and process for the production of IAD 1000 mg powder for oral solution in sachets is proposed. IAD solubility studies have shown that a dose of 1000 mg of IAD is easily dissolved in a glass of water before administration of the product. Spray-dried mannitol has been considered the most appropriate diluent for the preparation of the direct powder mix. The optimal parameters of the process of blending the powder components of the product are determined. The results of the stability study indicate that the sachets remain stable under the observed conditions. After reconstitution in water, the product provides a 100% active substance in the solution for 30 minutes.

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