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Full Length Research Paper

Comparison of the physical and chemical changes of magistral suspension with anesthesin and aethenamine after date of expiration

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This study reports the differences in physical and chemical stability of suspension with dissolved and non dissolved main active compound. In the first case it is methenamine and the other one is anesthesin. At the same time we analyzed two fresh and two 18-months old formulations of both magistral preparations. Anesthesin suspensions did not show any significant physical or chemical changes in 18 months except changes in appearance. On the other hand suspension with methenamine showed as very unstable system. Methenamine was hydrolyzed in water and ammonia and formaldehyde were determined as degradation products. Level of methenamine in preparations after 18 months was 84.6 ± 1.1%. Also the value of water was slightly decreased in old formulation with methanamine, which indicates a physical unstable system.

Key words: Methenamine, anesthesin, suspensions, magistral preparation stability.

INTRODUCTION

Suspensions are an important class of pharmaceuticals dosage form. These disperse systems present many formulations, stability, manufacturing and packaging challenges. Knowledge of the theoretical considerations pertaining to suspension technology should ultimately help the formulator to select the ingredients that will be most appropriate for the suspension and to use the mixing and milling apparatus available to the best advantage (Lachman et al., 1976).

The suspension must remain sufficiently homogenous for at least the period between shaking the container and removing the required dose. The sediment produced on storage must be easily re-suspended by the use of moderate agitation. The suspended particles should be small and uniformly sized in order to give a smooth, elegant product free from a gritty texture (Churchill, 1988).

Methenamine is powder (crystals, granules) which is in use as urinary antiseptic for oral administration and antiperspirant for external use (foot cream and suspension). 1 g dissolves in 1.5 ml water, 12.5 ml alcohol, 320 ml ether or 10 ml chloroform. pH value of 0.2 M water solution is 8.4 (The Merck Index, 1996). Hydrolysis of methenamine to ammonia and formaldehyde in water is slow (Petri, 2001).

Anesthesin is white powder commonly used as local anesthetic for topical preparation. 1 g dissolves in about 2500 ml water, 5 ml alcohol, 2 ml chloroform, 4 ml ether and in 30 to 50 ml of expessed almond oil or olive oil. Anesthesin is stable in air and has pKa 2.5 (The Merck Index, 1996).

Some studies have been published for stability of magistral suspensions, but none for suspensions with methanamine or anesthesin. Stability of suspension were developed for microcrystal of insulin (Kwon et al., 2004), microcrystalline -sitosterol in oil (von Bonsdorff-Nikander et al., 2003), with polysorbate 80 (Duro et al., 1998), heat-induced formulation in homogeneity of a three-component suspension (Toongsuwan et al., 2004), development of a topical suspension containing three active ingredients (Chang et al., 2002), rheological behaviour of nasal sprays (Eccleston et al., 2000), extem-poraneous norfloxacin suspension (Boonme et al., 2000), physical and chemical stability of niclosamide crystal forms (de Villiers et al., 2004) and formulation of a charcoal suspen-sion for intratumoral injection (Bonhomme-Faivre et al., 1999).

The purpose of this work study was to further characterize the stability and degradation mechanisms of metha-

Table 1. Formula for the preparation of methenamine and anesthesin suspensions.

Ingredients	Amount used	Amount used
Methenamine	10 g	/
Anesthesin	/	10 g
Zinc oxide	10 g	10 g
Talc	20 g	10 g
Glycerol	20 g	10 g
Water	up to 100 g	60 g

namine and anesthesin in pharmaceuticals, magistral suspensions for external use. Both formulations are on the top of the most prescribed in pharmacy made drugs during the summer period, either for adult or paediatric population. Swelling and perspiration of feet at the season with very high temperature could be a problem which can bring secondarily mycotic infections of nails and fingers. It could be prevented with appropriate using of magistral methanamine suspension. On the other hand, allergy, herpes, erythema, or some other painful and unpleasant skin diseases should be treated with different drugs and also with anesthesin to reduce pain and itchiness (Petri, 2001). According to statistical data around 50 - 60% of all world population has some of allergic reaction per year. Some of them are really distasteful (Weiss, 2004).

Aim of this work is to compare results of physical and chemical characteristics of fresh suspensions and 18 months old magistral (in pharmacy) made suspensions of methenamine and anesthesin. Tests of stability and expiration date for all kind of pharmaceuticals are given in different directives (Directive 2001/83/ES, 2001; Directive 2004/24/ES, 2004; EMEA/CVMP/846, 1999; ECEIDG, 2005; ICH Q1A(R2), 2003), but date of expiration for galenical and magistral suspensions are not determined. In practice it is usually 12 months at room temperature for stable form.

MATERIALS AND METHODS

Materials

All the chemicals were BP or EUR quality and were used without further purification. Anesthesin, methenamine, talc, zinc oxide and glycerol were obtained from Centrohem (Belgrade, Serbia). Suspensions were prepared with deionised water (Milli-Q-quality).

Preparation of suspensions

Suspensions of anesthesin (*Mixturae Aethylis Aminobenzoatis*) and methenamine (*Mixturae Methenamini*) were prepared according to prescription from Magistral Formula III (FM III Yugoslavia) (FM III, 1979).

A 100 g portion of each suspension was prepared according to formulas in Table 1. We were used three suspensions of each prescription for repeatability study (anesthesin and methenamine) prepared extemporaneously and 18 months before analysis. Each serial preparation was made by different pharmacist. Four pharmacists made three 100 g of portion (I – 3 x 100 g anesthesin suspension 18 months before analysis; II – 3 x 100 g anesthesin suspension made *ex tempore*; III – 3 x 100 g methenamine suspension made 18 months before analysis; IV - 3 x 100 g methenamine suspension made *ex tempore*).

Analysis of samples

We were analysed sedimentation volume, particle size changes, pH value, appearance of preparations, and quantification of each compound, identification potential new chemical degradation products, viscosity of excipient and variation of preparations weights.

Appearance of preparations was detected one hour after fresh suspensions were made. We compare colour, structure of sediment, re-suspending after shacking, foaming, smell and spilling of suspensions. The sedimentation volume (V_u/V_o) was measured after the suspensions were settled 48 h after preparation of fresh suspensions in a 25 ml graduated cylinder. Spontaneous sedimentation was measured at room temperature. V_u is the ultimate volume of the sediment and V_o is the volume of the suspension.

Determination of each compound and identification of degradation products were according to 5th European Pharmacopoeia (Ph. Eur. V, 2005).

The size and shape of particles were evaluated by optical microscopy (A. Kruss – Optronic, Germany) with colour video camera (Topica TO-1480, Croatia). The samples were prepared by taking a small amount of the suspension (1 ml) that was diluted, because of the high viscosity, with a small amount (9 ml) of 10 mM SDS in water. The size of 500 particles per sample was measured manually using a measuring rod.

A Metrohm 691 pH meter by Herisau (Switzerland) was used for pH measurement. pH meter was calibrated between each measurement with pH 7 and pH 10 buffers.

The viscosity of the excipient (liquid part) was determined theoretically according to spherical particle radius, densities and velocity of sedimentation by Stokes' law.

RESULTS AND DISCUSSION

Fresh, extemporaneously made, suspensions had better characteristics. More visual changes were detected for methenamine suspension then for anesthesin.

Results are present in Tables 2 and 3. Also variation of preparations weights was measured. Variation of preparation weight is not significant for suspension I, II and IV, but methenamine suspension made 18 months before analysis (IV) has 87.0 ± 3.9% of 100 g portion. It could be result of losing water from plastic packing system, as well as in water dissolved compound, methenamine, after chemical degradation (ammonia and formaldehyde). Sedimentation is slower for fresh suspensions, which is not typical for 18 months old suspensions (Table 3). Anesthesin suspension made 18 months before analysis has sedimentation volume 0.57, after 1 h, because there are many aggregates in that system. On the other hand old suspension with methenamine has in first hour sedimentation volume 0.98, which is higher then for same fresh disperse system (0.93). It is probably cause by losing water and viscosity of excipient with glycerol is higher. But, after 24 and 48 h sedimentation volumes are higher

Table 2. Visual examination of the samples.

Samples	I	II	III	IV
Colour	off white	white	grey	white
Sediment	flocculated	disperse	deflocculated	disperse
Re-suspending	good	very good	problematic	very good
Foam	no	no	stable foam	not stable
Smell	without	without	NH_3; CHCHO	without
Spilling	good	very good	problematic	very good

I - anesthesin suspension made 18 months before analysis; II – anesthesin suspension made extemporaneously; III – methenamine suspension made 18 months before analysis; IV - methenamine suspension made extemporaneously.

Table 3. Result of preparation weight and sedimentation volume.

Sample	Weights ± SD (%)	RSD (%)	Sedimentation volume		
			1 h	24 h	48 h
I	95.0 ± 1.3	1.4	0.57	0.39	0.39
II	95.1 ± 1.6	1.7	0.88	0.70	0.63
III	87.0 ± 3.9	4.5	0.98	0.65	0.59
IV	95.3 ± 1.7	1.8	0.93	0.75	0.72

I - anesthesin suspension made 18 months before analysis; II – anesthesin suspension made extemporaneously; III – methenamine suspension made 18 months before analysis; IV - methenamine suspension made extemporaneously.

for fresh suspension with methenamine, because aggregation is present in old one.

The pH of fresh suspension with anesthesin and methenamine was 8.0 ± 0.2 and 8.4 ± 0.1, respectively. Change of pH value for anesthesin was not significant, but for methenamine was slightly increased after 18 months (8.9 ± 0.2), because chemical degradation of methanamine. According to 5th European Pharmacopoeia (Ph. Eur. V, 2005) it was found that old methenamine suspensions showed positive identification reaction for ammonia and formaldehyde as impurities.

The samples of the suspension stored at room temperature for 18 months were examined under an optical microscope and compared to a freshly made sample. Photomicrographs of the fresh suspensions with methenamine and anesthesin (Figure 1a and 1c) showed a mean particle size of approximately 2 to 4 m and 1 to 5 m, respectively. 18 months old suspensions (Figure 1b and 1d) showed aggregation and a mean particle size were 2 to 5 m with a lot of small aggregates (different shapes; 10 m) and 1 to 5 m with few aggregates (differrent shapes; 8 to 12 m) for methenamine and anesthesin, respectively. As it can be seen, the particle size in suspension with anesthesin is the same in fresh and 18 months old suspensions, which indicate good physical stability of this system. On the other hand, a lot of aggregates, as well as a slightly growing of particle in suspension with methenamine after 18 months, indicate physical unstable suspension.

According to spherical particle radius, densities and velocity of sedimentation by Stokes' law, the viscosity of the excipient (liquid part) were determined theoretically. It was approximately 3.2 x 10^{-3} and 6.9 x 10^{-2} Pas for fresh and 4.3 x 10^{-3} and 7.1 x 10^{-2} Pas for old suspension with methenamine and anesthesin, respectively. Results for suspensions with methenamine improved slightly increasing of excipient velocity which was suspected during the determination of suspension volume and weight of preparation, as lost of water from the system.

Anesthesin, methenamine, talc, zinc oxide and glycerol were determined according to 5th European Pharmacopoeia (Ph. Eur. V, 2005). Recovery of water was defined calculating weight of preparation and sum of other compound from formulation. Results for all four formulations are shown in Table 4. Changes for old anes-thesin suspensions during 18 months are not significant according to results for all compounds in the same fresh formulation. In formulation with methenamine we found signifycant changes of methenamine and water. Value of methenamine in fresh suspensions was 99.2 ± 0.7 % but in 18-months old suspensions 84.6 ± 1.1 %. Ones more we conform that methenamine is chemically unstable in water and amount for its decreasing with time. In our case, methenamine was dissolved in water as on of the excipient, which cause hydrolyses, while zinc oxide and talc were suspended in excipient and they are stable.

Table 4. Recovery and standard deviation of each compound.

Sample	I Recovery [%]	RSD [%]	II Recovery [%]	RSD [%]	III Recovery [%]	RSD [%]	IV Recovery [%]	RSD [%]
Anasthesin	99.1 ± 3.1	3.1	99.3 ± 2.4	2.4	/	/	/	/
Methenamine	/	/	/	/	84.6 ± 1.1	1.3	99.2 ± 0.7	0.7
Zinc oxide	97.5 ± 1.9	2.0	99.5 ± 2.8	2.8	96.8 ± 2.0	2.1	97.2 ± 0.8	0.8
Talc	96.9 ± 0.9	0.9	96.0 ± 1.5	1.6	96.5 ± 1.2	1.2	97.3 ± 1.3	1.3
Glycerol	99.2 ± 0.7	0.7	99.5 ± 0.4	0.4	98.3 ± 0.7	0.7	98.5 ± 1.0	1.0
Water	97.8 ± 0.6	0.6	98.1 ± 0.3	0.3	93.9 ± 0.7	0.8	98.1 ± 0.9	0.9

I - anesthesin suspension made 18 months before analysis; II – anesthesin suspension made extemporaneously; III – methenamine suspension made 18 months before analysis; IV - methenamine suspension made extemporaneously.

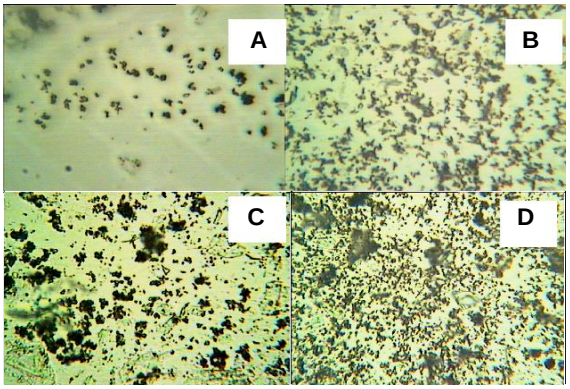

Figure 1. Photomicrograph of suspension **A**) methenamine (extemporaneously), **B**) methenamine (at room temperature for 18 months), **C**) anesthesin (extemporaneously), **D**) anesthesin (at room temperature for 18 months); 1 mm at picture is 1 m.

the value of water was slightly decreased in old preparation with methenamine, which is indicates a physically unstable system.

Conclusions

It could be concluded that a suspension containing methenamine has physical and chemical changes during the storage time. Ammonia and formaldehyde as degradation products can cause toxic effects after topical use of suspension when expired. Also we conformed that suspendsions with non dissolved compounds are more stable. Methenamine was dissolved and anesthesin was suspended which indicated different stability of two formulations after 18 months. According to our results anesthesin suspension could be in used more then one year after production, as it was usually in practice. That is not case with methanamine suspension for topical use prescribed according to FM III.

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