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Assessment of preservative efficacy of sodium N-hydroxymethylglicinate (NIG) in drug delivery formulations for ophthalmic use

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ABSTRACT

Different drug delivery systems, namely polymeric nanoparticles (NP), nanostructured lipid nanoparticles (NLC), and *in situ* gelling polymeric solutions (Gel), containing sodium N-hydroxymethylglycinate (NIG) at 0.002% (w/v) as a preservative agent were prepared as sterile formulations and characterized. The study aimed at verifying the maintenance over time of sterility conditions of these polymeric and colloidal systems, after exposure to environmental contamination. It is in fact known that many preservatives can interact with drugs and other additives in a formulation, or be adsorbed on the surface of plastic containers. The same phenomenon might occur when the formulation contains nanosized (colloidal) lipid or polymeric matters. Our experimental findings confirmed the capacity of NIG to keep the sterility of both fresh and packaged nanoparticulate or polymeric systems.

Keywords: *eye-drops; ophthalmic drug delivery system; colloidal systems; sterility; ophthalmic preservatives; european pharmacopoeia guidelines.*

1. INTRODUCTION

Ophthalmic drug delivery systems (ODDS) are used to administer drugs in anterior and posterior ocular areas, with the aim to increase the therapeutic efficacy of drugs, by prolonging their permanence on the ocular surface or improving or sustaining drug accumulation in ocular tissues.

Basing on the nature of components, ODDS can belong to vesicular systems (such as liposomes and niosomes), colloidal particulate systems (such as polymeric micro- or nanoparticles, nanocapsules, lipid-based nanoparticles), nanoemulsions, and also supramolecular systems (such as cyclodextrins inclusion complexes or polymer conjugates) [1-5]. The use of delivery systems presents various advantages in terms of increasing the local bioavailability of the carried drug and its stability, enhancing the penetration across ocular tissues and barriers, reducing systemic side effects, decreasing the number of administrations, promoting a targeted and controlled drug release [6-8].

One pharmacopoeial mandatory requirement for ophthalmic formulations is sterility; for multidose devices, the preservation from microbial contaminations after container opening and along the use of the medicine. Therefore, it becomes crucial to improving the biological stability of the formulation by adding preservative agents. Ophthalmic preservatives must possess stringent characteristics, such as to be safe and not irritating, efficient at low concentrations, compatible with the drugs and whole formulation [9, 10].

One of the preservatives very popular in ophthalmic formulations is N-hydroxymethylglycinate (NIG). It is derived from glycine amino acid and is very soluble in water and glycerine, but insoluble in alcohols and oils. Usually, the most common form of NIG is the sodium salt [11, 12]. NIG is compatible with anionic compounds, but incompatible with cationic substances. It can be used in a large pH range (from 3 to 12) and is very active against both Gram negative and Gram positive microorganisms, but shows a low activity against fungi [12]. It belongs to the chemical class of substances called 'formaldeheyde donor' and it acts releasing gradually formaldehyde, a toxic component for microbial populations [11, 12]. NIG has been sometimes cited as a dangerous component also for human health, but it can be actually used at low concentrations (MAX. 0.5% w/v) to ensure a safe margin of use [13]. Recently, the absence of toxicity of NIG *in vitro* at a concentration of 0.002% (w/v) in corneal cells has been confirmed [14].

It is a common observation that preservatives can interact with drugs and other ingredients of a formulation, or can become adsorbed on the inner surface of plastic containers [15-18]. This leads to a reduction of the available amount of preservative, with the risk of reaching a concentration below the levels of antibacterial activity. It is conceivable that the same phenomenon might occur when the formulation contains macromolecules or nanosized (colloidal) lipid or polymeric matters. Therefore, in the present study, we evaluated the efficacy of NIG in maintaining the sterility, upon storage or exposure to usage-like conditions, of liquid formulations containing either polymeric or lipid nanoparticles, or soluble polymers.

2. EXPERIMENTAL SECTION

Materials. Gelucire® 44/14 (lauroyl polyoxyl-32 glycerides) was donated by Gattefossé (Saint- Priest, France). Mygliol® 812 (triglyceride capric/caprylic acids) was purchased from Farmalabor s.r.l. (Assago, Italy). Tween® 80 (polysorbate 80),

NIG, Cremophor® A25 were purchased from Sigma-Aldrich srl (Milan, Italy). Eudragit® RS100 and RL100 (Evonik Nutrition & Care GmbH, Essen, Germany) were kindly provided by Rofarma Italia srl (Gaggiano, Italy). Gellan gum was purchased from Thermo Fisher Scientific (Karlsrube, Germany). Simulated tear fluid (STF) was prepared containing 200 mg of sodium bicarbonate, 670 mg of sodium chloride and 8.3 mg of dihydrate calcium chloride in 100 ml of water for injection (corresponding to 1.11 mEq/L calcium, 142 mEq/L sodium, 116.7 mEq/l chloride, 27.2 mEq/L bicarbonate) [19]. The pH was adjusted to 7.4 using 0.1 N HCl or NaOH.

Methods.

Preparation of the colloidal systems. The composition of the studied lipid and polymeric systems is given in Table 1.

Nanostructured lipid carriers (NLC; batch FR1) were produced by a melt-emulsification and ultrasonication technique [20]. A lipid mixture matrix based on a solid lipid (Gelucire® 44/14) at a concentration of 10% (w/v), and a liquid lipid (Mygliol® 812), at a concentration of 3% (w/v). This lipid mixture was melted at 75-80°C. An aqueous phase containing Tween 80 (2.5%, w/v) as a surfactant and NIG (0,002% w/v) in distilled water was warmed to the same temperature and then slowly injected into the lipid phase under magnetic stirring, along 20 min. The resulting pre-emulsion was put in an ice bath and sonicated with a probe sonicator (Branson 102c, Bransonic, USA; duty cycle 70, output control 6) for 15 min. At the end, the obtained formulation was stored in a refrigerator and analyzed after 24 h.

Polymeric nanoparticles (NPs; batch FR2) were made with Eudragit® Retard resins, a family of methacrylic and acrylic acid copolymers, with low ammonic quaternary groups [21]. The NP was prepared using the Quasi-emulsion solvent diffusion (QESD) method [22-25]. Briefly, Eudragit RS100 and RL100 (in a 1:1 weight ratio) were dissolved overnight in 20 ml of ethanol to prepared an organic phase. Two ml of the polymer solution were slowly injected (along 15 min) through a thin Teflon tube connected to a plastic syringe into 15 ml of the aqueous phase (solution of 0.05% (w/v) Tween® 80 in water for injections). The mixture was kept under high-speed stirring (15,000 rpm) using an Ultra-Turrax T 25 homogenizer (IKA®-Werke GmbH & Co. KG, Staufen, Germany) for 30 min in an iced water bath at 0 °C. In the end, the obtained mixture was stirred for 24 h to allow the complete evaporation of the solvent. NIG (0.002%, w/v) was finally added to the formed nanoparticle suspension.

Table 1. Compositions (%, w/v) of the tested systems.

	Ingredients	FR1	FR2	FR3
Aqueous phase	NIG	0.002	0.002	0.002
	Tween 80	2.5	0.5	-
Lipid matrix	Mygliol 812	3		
	Gelucire 44/14	10		
Polymeric phase	Eudragit RS100		0.05	
	Eudragit RL100		0.05	
Gelling system	Gellan gum			0.3
	Cremophor A25			0.3

The *in situ* gel forming system (batch FR3) was prepared by warming at 40 °C a solution of NIG 0.002% by weight in water for injections. Cremophor® (0.3%, w/v) as a surfactant and gellan gum (0.3%, w/v) as the gelling agent was added and mixed at 40 °C for 1 h to create a homogeneous phase. The formulation was finally cooled down to room temperature and stored at room conditions in closed glass vials.

Characterization of the produced formulations

Size analysis. Mean particle size (Z-Ave) and polydispersity index (PdI) were determined by photocorrelation spectroscopy (PCS) using a NanoSizer ZS90 (Malvern Instruments, UK). Samples were diluted ten-fold with water before analysis; given values are the mean ± SD of 3 series of 10 measurements in triplicate (90 measurements).

Gelling studies. The gelling ability of the *in situ* forming gels was evaluated using STF (pH 7.4), containing the same ions present in natural tears and necessary for the sol-gel transition of gellan gum [26]. One ml of the formulation was mixed with 200 µl of STF in a glass test tube and any gelation phenomenon was registered.

The permanence in the gel state was evaluated by tilting the test tube at 90° and observing the time at which the gel became again fluid and flowed away.

Sterilization. The work material, in accordance with the requirement of sterility, mandatory for the preparations of ophthalmic products, was subjected to biological steam sterilization using a Panasonic MLS 2420U bench-top autoclave. To ensure the sterility of all the material used, both the containers used for the formulations (20-ml amber glass bottles) and the respective closure caps were subjected to the same procedure. They were individually wrapped in aluminum foil, then covered with wrapping paper in groups of 4 units. The packages were placed first in a large glass beaker and then inside the autoclave basket. The sterilization was carried out at a pressure of 1 atm and a temperature of 120.5 °C for 20 min. Once the automatic sterilization cycle was completed, the sterilized material was first allowed to depressurize and then unwrapped, operating under aseptic conditions in a sterilizing hood.

Sterilization of the lipid and polymeric systems. NP was sterilized by filtration, under aseptic conditions in a sterilizing hood, through 13-mm Whatman® GD/X cellulose acetate sterile filters (Thermo Fisher Scientific, Rodano, Italy) with a pore size of 0.2 µm into the previously autoclaved sterile containers.

In accordance with the provisions of the sterility test of the Italian Pharmacopoeia [27] a number of 1-ml samples of not less than 4 (named as A, B, C, D) were prepared for each formulation to be tested and for each predetermined time interval (time 0; 15 days; 60 days). In addition, a control specimen consisting of water (20 ml) and NIG (0.002%, w/v) (batch FR0) was sterilized by filtration and sampled. The whole operation was very delicate and was carried out scrupulously in order to avoid the pollution of the material.

Sterility analysis. According to the test proposed by the Italian Pharmacopoeia [27], the formulations were assayed to evaluate the possible presence of various microorganisms such as aerobic bacteria, anaerobes, and fungal species. The first step of the method, according to European Pharmacopoeia, 9th Edn. [28], was the dilution, inside test tubes, of 1 ml of each sterilized

formulation with 9 ml of BHI (Brain Heart Infusion Broth (Oxoid Ltd., Thermo Fisher Scientific, Rodano, Italy) broth. Each tube was thereafter incubated at 37°C under aerobic and anaerobic conditions for about 15 days in order to highlight any microbial growth. Then, aliquots of each tested sample were seeded in Muller-Hinton and Sabouraud Dextrose agar (Oxoid Ltd., Thermo Fisher Scientific) and incubated for 72 h at 37°C and 33°C, respectively.

3. RESULTS SECTION

Characterization of the formulations. The produced NLC (FR1) and NPs (FR2) were analyzed in term of Z-Ave and PdI (Table 2).

Table 2. Z-Ave and PDI values (± S.D.) of FR1 and FR2 nanoparticles.

Batch	Z-Ave	PDI
FR1	42.7 ± 1.15	0.404 ± 0.015
FR2	63.0 ± 2.10	0.661 ± 0.03

Both the nanoparticle systems showed a very low particle diameter, well below the value (200 nm) considered suitable for the ocular application.

The polymeric solution FR3 showed to be able to form a stable gel phase upon contact with mono- and bivalent ions (STF) at room temperature, due to the presence of gellan gum (Pignatello et al., unpublished results). The system remained in a gel state for up to 1 h at room conditions (Figure 1).

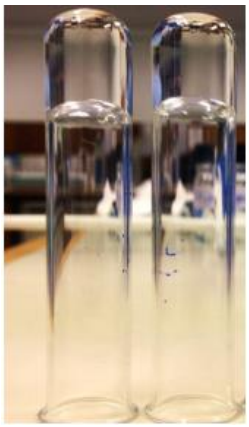

Fig. 1. Evidence of gelation of the gellan gum-based formulation (batch FR3) in the presence of STF: time= 5 min (left) and 1 h (right).

Evaluation of the preservative activity of NIG. The growth inhibitory capacity of NIG against fungi and gram positive and gram negative bacteria in sterile colloidal samples was evaluated through the study of preservation of sterility conditions, at established time intervals:

- immediately after sterilizing filtration (time 0);
- after exposure of sterile samples to environmental conditions for 15 days (time 15 days);
- after storage of sterile samples in closed containers for 2 months, followed by the opening of the packages and exposure for 15 days at environmental conditions (time 60 days).

The values obtained from the analysis are shown in Table 3:

Table 3. Evaluation of the conservative power of NIG (0.002 %, w/v) in different formulations in a range time 0-60 days.

Sample	Time (day)	Formulation	Microbiological result
FR 0	0	Water	No growth
FR 0	15	Water	No growth
FR 0	60	Water	No growth
FR 1	0	NLC	No growth
FR 1	15	NLC	No growth
FR 1	60	NLC	No growth
FR 2	0	NP	No growth
FR 2	15	NP	No growth
FR 2	60	NP	No growth
FR 3	0	Gel	No growth
FR 3	15	Gel	No growth
FR 3	60	Gel	No growth

Concerning the microbiological aspects, all the examined formulations at all the tested times did not show the growth of any microorganism in the used culture media, both in broth and subsequently in agar plates.

4. CONCLUSIONS

The experimental assays showed that all the systems maintain a good microbiological stability over time, without any presence of microbial growth both immediately after sterilizing filtration and after exposure to environmental conditions for 15 days. Preservation of sterility was also verified after keeping the samples in closed containers for 2 months, followed by opening the packages and exposure for 15 days to environmental conditions.

The antimicrobial activity of NIG, at the concentration of 0.002% (w/v), commonly used in pharmaceutical and medical devices, in maintaining the sterility of nanoparticulate or polymeric systems was thus fully confirmed.

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