HYDROLYTIC STABILITY OF DILTIAZEM IN THE PRESENCE OF CHOSEN POLYMERS - POLOXAMER 407 AND AMMONIO METACRYLATE COPOLYMER TYPE B (EUDRAGIT RS): A PRELIMINARY STUDY

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ABSTRACT

INTRODUCTION: The hydrolytic stability of the calcium channel blocker representative *diltiazem hydrochloride* in the presence of different polymeric entourage was quantitatively assessed using HPLC.

AIM: The experiment was performed in order to establish drug-excipients compatibility in *Poloxamer 407* hydrogel formulations, where the drug was introduced either by a direct (conventional) approach or in the form of *Eudragit RS microsphere*-type particles with sustained drug release.

MATERIALS AND METHODS: Samples of conventional *diltiazem* 2% hydrogel and *microsphere*-enriched *diltiazem* 2% hydrogel were stored at 25±2°C for 10 months, protected from light. Analysis of the chemical decomposition rate of *diltiazem hydrochloride* to its *desacetyl* degradant - *O-desacetyl-diltiazem hydrochloride* - was performed in the period between 4th and 10th month of storage.

RESULTS AND DISCUSSION: It was hypothesized that a polymeric “shield” of *Eudragit RS* in the composition of *diltiazem*-loaded porous microspheres and/or the enhanced viscosity achieved by the addition of high-molecular gelling agent *Poloxamer 407* to an aqueous drug dispersion will benefit the drug’s stability. Instead, a negative effect on the hydrolysis rate was found to be dominant for both polymers, likely due to an “unfavorable” shift in the dielectric conductivity of the media and/or suspected catalytic effect of quaternary ammonium groups of *Eudragit RS*.

CONCLUSION: These preliminary results led us to a deeper understanding of the polymeric impact on *diltiazem* hydrolytic behavior and a very useful foundation for a future preformulation stage in the development of *diltiazem* modified-release semi-solid forms for the treatment of chronic anal fissure.

Keywords: *diltiazem* hydrolysis, *diltiazem* stability, drug-excipient compatibility, polymeric drug delivery, HPLC

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INTRODUCTION

The chemical stability of an active pharmaceutical ingredient is of critical value for the dosage forms endurance and shelf life. Hydrolysis, as a common process leading to drug decomposition, affects primarily ester, amide, imide, thioester, lactam, and lactone functional groups. The kinetics and mechanism of the hydrolytic process depend on intrinsic (related to the nature of the drug compounds) and external (environmental) factors. Important compound-related factors are the type of hydrolytically unstable bond (e.g., ester, amide etc.), electrophilicity of the carbonyl C-atom, substituents by and around the same, steric stabilization, potential for intramolecular catalysis, ionizability, ability of the leaving group (e.g., alcohol), polymorphism and reactive surface area (drug particle size). The most important among external factors are the temperature, pH, moisture/water content, water mobility, ionic strength, presence of catalytically active ions and functional groups (intermolecular catalysis), dielectric constant, and type of drug dispersion (e.g., a solution or a suspension). All variables belonging to the group of environmental factors (with the exception of temperature) may be a function of the excipients used in a dosage form (1).

It was of our interest to follow the impact of two commonly used polymers in the pharmaceutical industry - Poloxamer 407 and ammonio metacrylate copolymer Type B (Eudragit RS) on the hydrolytic degradation of *diltiazem hydrochloride* – a vasodila-

tor of the calcium channel blocker group - in hydrogel dispersions.

The latter are applicable in the treatment of chronic anal fissure (CAF), but are most often prepared *ex tempore*, given the low stability of the compound in a hydrated environment and respective short shelf life.

Essential for the hydrolytic degradation of *diltiazem* is its acetyl oxide radical (2). Hydrolysis of the CH_3COO -radical results in the formation of equimolar amounts of *O-desacetyl diltiazem* (des-*diltiazem*) and *acetic acid* (Fig. 1) (3,4). Des-*diltiazem* possesses only quarter to half of the pharmacological activity of the parent drug molecule (5,6). The relatively high hydrolytic stability of the 7-membered lactam ring of *diltiazem*, on the other side, is likely due to a steric stabilization. Maximal stability and lowest rate of hydrolysis of *diltiazem hydrochloride* are reported at low-pH values in the range 3-5 (1,4,7).

Fig. 1. Hydrolysis of *diltiazem* to des-*diltiazem*

In addition, under the influence of UV-Vis electromagnetic radiation, *diltiazem* is found to epimerize reversibly to its (+)-(2R, 3S)-trans *diltiazem* antipode, owing significantly lower coronarodilator ability (8,9). The photoinstability of *diltiazem* should be considered when choosing a primary packaging for the substance in bulk and *diltiazem* containing dosage forms (1).

The high reactivity and low hydrolytic stability of *diltiazem* occurs both *in vitro* (during storage) and *in vivo* (after intake). The latter manifests as low resistance to esterases and other enzymes (10). Respectively, *diltiazem* owns a short biological half-life ($t_{1/2}$) and oral bioavailability (F) ($t_{1/2}$ 3-4.5 h; F ≈ 40%) (4-6). In this view, there is a pharmacokinetic prerequisite to formulate *diltiazem* in modified-release dosage forms. The same applies when it comes to rectal *dil-*

Diltiazem hydrochloride

Hydrochloride of (2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl **acetate**

Scheme 1. Structure of *diltiazem hydrochloride*

tiazem dosage forms. In this case, the sustained drug release overcomes some drawbacks showed by the conventional topical *diltiazem hydrochloride* formulations for the treatment of CAF. A sustained drug delivery form of *Eudragit RS microsphere*-based *Poloxamer diltiazem* 2% hydrogels demonstrated a better drug deposition in rectal mucosa and lower absorption, thus declaring the potency to reduce anal pruritus and postural hypotension caused by fast dissolution and absorption of *diltiazem hydrochloride* (11).

AIM

The aim of the current survey was to assess the impact of the used polymers – *Eudragit RS* and *Poloxamer 407* – on *diltiazem* with respect to its hydrolytic stability.

MATERIALS AND METHODS

1. Materials – Chemicals and reagents were obtained from (as follows): *diltiazem hydrochloride* 99.9% - Puho Pharmaceuticals Co. Limited, China; *desacetyl diltiazem hydrochloride* – LGC GmbH, Germany; *ammonio methacrylate copolymer* (type B) (*Eudragit RS 100*) - Evonik Industries AG, Germany; *Poloxamer 407* (*Lutrol F-127*) – BASF, Germany; *Ethanol Absolute* HPLC grade and *Methanol* HPLC grade – Fisher Chemical, Germany; *orthophosphoric acid* > 85% - Fisher Scientific, UK.

2. Preparation of *diltiazem* 2% gels – two types of *diltiazem* 2% gels were prepared, using gel base *Poloxamer 407* 20%:

1. *Diltiazem* 2% *Poloxamer* 20% hydrogel (Px-Dtz-gel), where the drug was introduced in the gel base via water solution (direct approach) and

2. *Microsphere*-enriched *diltiazem* 2% *Poloxamer* 20% hydrogel (*micro*-Px-Dtz-gel), where the drug was introduced in the gel base via *diltiazem*-loaded *Eudragit RS 100 microsphere*-type particles.

Reference for the descriptive preparation techniques (for the gels formulation and the synthesis of *diltiazem*-loaded *microsphere*-type carriers) is available in our previous works (11-13). Sample of each formula was stored at 25±2°C in the dark for a period of 10 months. A standard *diltiazem* 2% solution in distilled water was used as reference; it was stored and tested under the same conditions.

3. Preparation of standard solutions – standard stock solutions of *diltiazem* and *des-diltiazem* were prepared in *methanol* at concentration of 100 µg/mL. Dilutions were performed by mixing both stock solutions in gradient percent ratios from 0% to 100% by a step of 10%. Standard calibration curves of *diltiazem* and *des-diltiazem* were built by using HPLC data.

4. Preparation of test solutions - 0.500 g of each test gel was dissolved in sufficient volume of *methanol* and further diluted up to 25.0 ml in a volumetric flask. Proper dilution with the same solvent was carried, where it was necessary, to obtain a theoretical concentration in the calibration range. The reference *diltiazem* 2% water solution was also analyzed after proper dilution, only with distilled water.

5. Analytical procedure – HPLC analysis – the stability tests were assayed by a Thermo Scientific UltiMate 3000 Analytical LC System, coupled with a Thermo Scientific Dionex UltiMate 3000 VWD-3100 Variable Wavelength Detector, following the procedure validated by Sadeghiet *et al* (3). The column – a Thermo scientific AQUASIL C18 (150 mm x 4.6 mm, 5 µm) and guard column - AQUASIL C18 (10 mm x 4.6 mm, 5 µm) - were settled at 50°C. The test samples were introduced in a volume of 10 µL. The mobile phase (*ethanol*: *aq. phosphoric acid* with pH 2.2 - 35:65) was injected in an isocratic mode with a constant flow rate of 1.2 mL/min. The total run time was 8 minutes. The detector was set at 238 nm. The peak areas were integrated automatically with the aid of a Thermo Scientific™ Chromeleon™ 7.2 Chromatography Data System software.

RESULTS AND DISCUSSION

The chromatograms obtained with *des-diltiazem* standard solution (100 µg/mL) and *diltiazem* 75 µg/mL: *des-diltiazem* 25 µg/mL mixture are shown on Fig. 2. Retention times for *des-diltiazem* and *diltiazem* were found at 2.6 min and 3.3 min, respectively. Standard curves for both compounds, as described above, were obtained in the concentration range of 10-100 µg/mL (Fig. 3).

Fig. 2. Chromatograms of a) des-diltiazem standard solution 100 µg/L and b) diltiazem 75 µg/mL: des-diltiazem 25 µg/mL mixture

Fig. 3. Standard calibration curves of a) diltiazem and b) des-diltiazem

Fig. 4. Chromatograms of test formulations obtained at 4th, 6th and 10th month of storing

Fig. 5. a) Increase in des-diltiazem concentration (% degradation) with time in test formulations; b) linear regression of the hydrolytic degradation using the data between the 4th and the 10th month of the study

The observed test samples showed substantial-ly different degradation rates of *diltiazem*, decreasing in the order *Poloxamer* 20%/ *Eudragit RS*-based hydrogel (*micro-Px-Dtz-gel*) > *Poloxamer* 20% hydrogel (*Px-Dtz-gel*) > water solution. Fig. 4 presents the chromatograms obtained for each *diltiazem* dispersion, following the decrease in the parent drug peak area and the increase in the degradation product – des-*diltiazem* – peak area with the time. Under the studied conditions, no other degradation products of *diltiazem* were detected.

The change in the des-*diltiazem* content as a function of time for each dispersion is shown graphically on Fig. 5a. When *diltiazem* was introduced in a *Poloxamer* gel, it showed faster initial decomposition rate compared to its aqueous solution. Knowing the relatively great chemical inertness of the hydrogel polymer (Fig. 6a), such escalation of the hydrolysis process could be only explained with the shift in the dielectric conductivity of the media. Further decomposition acceleration was observed when the drug was included in the hydrogel via *Eudragit RS 100* sustained-release microparticles. *Eudragit RS*, also known with its pharmacopoeial name *ammonio methacrylate copolymer Type B*, contains 4.5 to 7.0 per cent quaternary ammonia 2-(trimethylammonio) ethyl 2-methylpropenoate groups (Fig. 6b) (14). Being positively charged, *Eudragit RS* could be perceived as potential catalyst of the *diltiazem* hydrolytic degradation (1). The net negative effect on *diltiazem* stability was demonstrated to be only initial. After the 4th month of storage, a linear steady-state increase in des-*diltiazem* concentration was reached. The kinet-

ic constant (k) of the process is lowest for the *micro*-Px-Dtz-gel formulation (0.48% increase in des-*diltiazem* concentration per month) and highest for the aqueous solution (0.55%/month) at this stage (Figure 6b). The observed phenomena is likely to be a result of a prevail of a viscosity based stabilization and a polymer “shield” determined stabilization after the 4th month.

Fig. 6. Chemical structure of a) Poloxamer 407 and b) Eudragit RS

CONCLUSION

The complex kinetics of *diltiazem* hydrolysis in *Poloxamer* 407 gels suggest the presence of more than one factor with impact on the process. Usually, when this is the case, the concentration of the hydrolytic product increases in a polymodal manner, which turns the mathematical extrapolation of the experimental data in a complicated task. In our case, the polymodality of the des-*diltiazem* curves could be explained with the role of the polymer ingredients in the systems. The polymers – *Poloxamer* 407 and *Eudragit* RS - play a role of both - drug carriers and participants in the phase distribution, transition and dispersity of *diltiazem*. Hence, their physicochemical properties (crystallinity, stability, solubility, gelation, cross-linkage, etc.) and affinity to the drug molecule will be the factors determining the rate and order of

diltiazem hydrolysis. Again, the intermolecular catalysis is not excluded, especially in the case of *Eudragit* RS-enriched gels.

Despite the lack of complete straightforwardness in the results, the main conclusions to be drawn are:

1. *Diltiazem* possesses complex decomposition kinetics in *Poloxamer* 407 hydrogels (with or without *Eudragit* RS microsponges); likely due to the influence of multiple factors on the hydrolysis process, related to the impact of drug nature and concentration, polymer participation and phase distribution.
2. The enhanced viscosity of hydrogels was not found to benefit the hydrolysis stability of *diltiazem*.
3. The incorporation of *diltiazem* in a sustained-release microsphere drug delivery system composed by *Eudragit* RS did not demonstrate the expected protection effect against the environmental hydrates. This observation was explained with the possible catalytic effect of cationic *Eudragit* RS centers on the hydrolysis of *diltiazem*.

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