Kinetics of the Esterification of Active Pharmaceutical Ingredients Containing Carboxylic Acid Functionality in Polyethylene Glycol: Formulation Implications

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ABSTRACT: Polyethylene glycols (PEGs) are attractive as excipients in the manufacture of drug products because they are water soluble and poorly immunogenic. They are used in various pharmaceutical preparations. However, because of their terminal hydroxyl groups, PEGs can participate in esterification reactions. In this study, kinetics of two active pharmaceutical ingredients, cetirizine and indomethacin possessing carboxylic acid functionality, has been studied in PEG 400 and PEG 1000 at 50°C, 60°C, 70°C, and 80°C. HPLC–UV was applied for the determination of concentrations in the kinetic studies, whereas HPLC–MS was used to identify reaction products. The esterification reactions were observed to be reversible. A second-order reversible kinetic model was applied and rate constants were determined. The rate constants demonstrated that cetirizine was esterified about 240 times faster than indomethacin at 80°C. The shelf-life for cetirizine in a PEG 400 formulation at 25°C expressed as $t_{95\%}$ was predicted to be only 30 h. Further, rate constants for esterification of cetirizine in PEG 1000 in relation to PEG 400 decreased by a factor of 10, probably related to increased viscosity. However, it is important to be aware of this drug–excipient interaction, as it can reduce the shelf-life of a low-average molecular weight PEG formulation considerably. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2424–2433, 2014

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INTRODUCTION

An important part of preformulation constitutes studies of drug–excipient interactions, where the potential degradation of an active pharmaceutical ingredient (API) in combination with excipients is investigated.[1–3] Excipients are usually selected from the GRAS (Generally Recognized As Safe) list[4] and generally considered to be biologically inactive. However, they can react chemically with the API causing a decrease in content of API and formation of inappropriate reaction products between API and excipient. Such reaction products may, in worst case, lead to adverse effects in patients.

Polyethylene glycol (PEG) is used in many pharmaceutical dosage forms, for example, sustained-release formulations, film-coated tablets, ointments, and suppositories. PEG is attractive from a formulation perspective, as it is non-toxic and water-soluble. Furthermore, PEG is not found to be immunogenic itself, and antibodies to PEG can only be generated in combination with highly immunogenic proteins.[5] Since the first investigations of PEGylation[6] (covalent attachment of PEG to, e.g., proteins, peptides, and enzymes), a number of commercial products have emerged from the research performed on PEG-conjugated biomacromolecules.[7] However, so far, commercial products where PEG has been conjugated to small molecules have not been marketed.[8] Instead, there are scattered examples in the literature, where the use of PEG as an excipient shows a negative impact on the stability of the API concerning the formation of reaction products between API and reactive peroxides found in PEG.[9–12] In addition, a few investigations have been published concerning an esterification reaction involving PEG and APIs containing carboxylic acid functionality.[13,14] If PEG esters are formed in a formulation, they could theoretically be converted back into the parent compound because of hydrolytic and/or enzymatic cleavage in the human body. However, it has been observed with ester pro-drugs that the regeneration to the parent compounds does not always occur, because esterases possess certain structure–activity relationships.[15] Further, the safety of a formulation containing PEG esters is not known, and could potentially lead to adverse effects. If the safety of PEG esters has been found to be acceptable, PEG could be a possible option for a suppository formulation because of its favourable water solubility and melting point. Therefore, to predict shelf-life of the drug product in formulations containing PEG, it is of great importance to be able to determine the rate of formation of PEG esters in a given formulation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are formulated and marketed in several countries as suppositories for pain treatment of patients with difficulties in swallowing a tablet. The NSAID indomethacin is formulated as a suppository. The elevated temperature applied during the production process of suppositories containing PEG and carboxylic acid-containing API possibly facilitates nucleophilic attack from the terminal primary hydroxyl group in PEG molecules on the carbonyl group of the carboxylic acid resulting in ester formation. Esterification of indomethacin has been reported after long-term storage of commercial indomethacin suppositories

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containing PEG 300.[16] Thus, lower-average molecular weight PEGs as PEG 400 are sometimes incorporated into suppositories in proportions of 30%–70%. PEG 1000 can be used in proportions of 40%–75%.[17] In a recent study, PEG adducts between cetirizine or indomethacin and PEG 400 were observed after 5 days of incubation at 80°C.[14] The PEG adducts were separated and identified using supercritical fluid chromatography (SFC) hyphenated to mass spectrometry (MS). A few studies exist, where the kinetics of esterification with ethylene glycol has been studied.[18–20] With the use of different kinetic models, activation energies for esterification were determined in a range from 32 to 65 kJ/mol. However, to our knowledge, an in-depth kinetic study of the esterification of APIs in higher-average molecular weight glycols, for example, PEGs, does not exist at the moment.

The present study was undertaken to identify reaction products of the model compounds cetirizine and indomethacin in formulations representing suppositories containing either PEG 400 or PEG 1000. Reaction kinetics of cetirizine or indomethacin in PEG 400 as well as in PEG 1000 was evaluated in accelerated stability studies. Esterification of API and the concomitant formation of impurities, including degradation products formed from indomethacin hydrolysis, have been studied. The shelf-life at 25°C for PEG 400-containing formulations was estimated and verified for cetirizine. Cetirizine was selected as a model compound representing an API containing highly reactive carboxylic acid functionality.[14,21] The reactivity of indomethacin was studied because marketed indomethacin suppositories, Indocid®, contain PEG 4000 and PEG 8000.[22]

EXPERIMENTAL

Chemicals and Solvents

Cetirizine dihydrochloride, 4-chlorobenzoic acid, [5-methoxy-2-methyl-1H-indol-3-yl] acetic acid, and PEG 400 were obtained from Sigma–Aldrich Chemie (Steinheim, Germany). Indomethacin was obtained from Hawkins Inc. (Minneapolis, Minnesota). PEG 1000 (Ph.Eur. quality) was obtained from Fagron Nordic A/S (Copenhagen, Denmark). Formic acid and dimethyl sulfoxide (DMSO) was from Merck (Darmstadt, Germany). Methanol and acetonitrile (HPLC gradient grade) was from VWR (Copenhagen, Denmark).

Instrumentation

HPLC was carried out using an Agilent Technology 1200 series HPLC system (Waldbron, Germany) equipped with a G1379B on-line degasser, a G1312B binary pump, a G1316B column oven, a G1367C HiP-ALS-SL autosampler, and a G1315C photodiode array detector.

An Agilent Technology 1100 series (Waldbronn, Germany) G1978A 1100/MSD LC/MS instrument equipped with a G1379A on-line degasser, a G1312A binary pump, a G1316A column oven, a G1313A ALS autosampler, and a G1315B photodiode array detector was used for HPLC–MS. An ion trap LCQ Deca mass spectrometer was coupled to the HPLC system and operated with electro spray ionization in positive and negative mode using the following parameters: capillary voltage 4000 V, drying gas flow 16 L min^{-1}, drying gas temperature 270°C, and vaporize temperature 150°C. The scan range was from 300 to 1000 amu for reaction mixtures containing PEG 400 and from 500 to 2000 amu for reaction mixtures with PEG 1000. For identification of the hydrolysis products of indomethacin, a scan range of 100–500 amu was applied.

HPLC Analysis

A reversed-phase gradient HPLC method was developed. The mobile phases were: A: 0.1% (v/v) formic acid and B: 0.1% (v/v) formic acid in acetonitrile. The gradient elution profile with respect to mobile phase B was: 0–1 min: 1% → 10%; 1–4 min: 10% → 20%; 4–10 min: 20%; 10–15 min: 20% → 30%; 15–30 min: 30%; 30–40 min: 30% → 40%; 40–50 min: 40% → 60%. Column temperature: 30°C. For quantification in the study of reaction kinetics, an Agilent Eclipse Plus C_{18} column (50 × 2.1 mm, 1.8 μm) was used, and the flow rate was kept at 0.3 mL min^{-1}. The described chromatographic conditions were also used for the HPLC–MS method. For preparative chromatography, an Agilent Eclipse Plus C_{18} column (50 × 4.6 mm, 1.8 μm) was applied and the flow rate was increased to 1 mL min^{-1}.

Calibration standard curves were used for the quantification of cetirizine and indomethacin. Regarding indomethacin, the formed hydrolysis products were determined with calibration standards of 4-chlorobenzoic acid and [5-methoxy-2-methyl-1H-indol-3-yl] acetic acid. As the UV spectral profiles of the parent compounds and their PEG esters were identical, the same molar absorptivity was used for the determination of the concentrations of API and PEG ester.

The suitability of the HPLC method with UV detection was tested by linearity and limit of quantification (LOQ) measurements. Linearity was found acceptable within the selected concentration range of 0.2–3.0 mM for cetirizine and indomethacin. LOQ was found to be 0.59 μM for cetirizine and 0.45 μM for indomethacin. The sensitivity of the method was considered to be adequate for quantifying PEG esters in the prepared reaction mixtures.

Studies of Reaction Kinetics

Reaction mixtures containing PEG 400 (2.8 M) and cetirizine or indomethacin, $[A_0]$, with molar drug–excipient ratios of 1:20 ($[A_0] = 0.14$ M) or 1:100 ($[A_0] = 0.028$ M) were prepared. Additionally, reaction mixtures with PEG 1000 (0.80 M) were made with cetirizine or indomethacin ($[A_0] = 0.040$ M). The selected concentration of the APIs is considered to be representative for commercial suppositories. All reaction mixtures were made in triplicate. Cetirizine dihydrochloride was almost insoluble in PEG. However, to find a suitable kinetic model for the data, cetirizine was predissolved in DMSO resulting in a final concentration of 10% (v/v) DMSO. Indomethacin was dissolved directly in PEG. The reaction mixtures were incubated in glass vials of Type I,[23] closed with rubber stoppers and sealed with aluminum closures before being placed in ProBlot™ Hybridization ovens with a temperature variability of ±0.5°C. The temperatures for the reaction mixtures containing PEG 400 were chosen to be 323 K (50°C), 333 K (60°C), 343 K (70°C), and 353 K (80°C). The temperatures were selected to obtain accelerated stability data and generate an Arrhenius plot. In addition, a temperature range between 50°C and 60°C is normally applied in the manufacture of suppositories. Reaction mixtures containing PEG 1000 were only investigated at 353 K (80°C). At definite time intervals, samples were withdrawn, cooled to room temperature, mixed on a whirl mixer, and diluted 50 or 100 times in either water (cetirizine) or in equal parts (v/v)