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Research paper

Contamination of semi-solid dosage forms by leachables from aluminium tubes

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ABSTRACT

The objective of this study was to determine to what extent bisphenol A (BPA), bisphenol A diglycidyl ether (BADGE) and its derivatives are extractable from epoxy-based coatings of aluminium tubes for pharmaceutical use and to monitor their leaching into different kinds of semi-solid dosage forms. Migration increasing factors should be evaluated. Extraction tests using acetonitrile for 10 days at 40 °C turned out to be suitable to estimate the maximum amount of extractables. A plain variability in the nature and amount of extractables among tubes of different vendors ($n = 7$) could be demonstrated. Leaching of the remnants into various semi-solid drug products (ointment, cream, gel) during storage (30 °C/40 °C) was verifiable. Leachable profiles were, apart from storage time and temperature, decisively influenced by the matrix. In particular, matrix polarity seemed to play a crucial role. Thus, the highest amount of leachables was found in isopropanol-based carbomer gel. Furthermore, in-use conditions (mechanical stress) enhanced migration significantly. In order to ensure quality and safety of semi-solid formulae, interactions between the coating material and the drug product should be thoroughly evaluated.

1. Introduction

The aluminium tube is the most commonly used container closure system for semi-solid dosage forms combining high barrier properties with simple handling. The tubes are internally lacquered to avoid direct contact between medicinal products and metal, which might lead to corrosion and migration of metals. The protective coatings usually consist of epoxy resins known for their good mechanical properties and chemical resistance. Since use of bisphenol F diglycidyl ether (BFDGE) and novolac glycidyl ethers (NOGE) in the production of lacquers was prohibited in 2005 [1], bisphenol A diglycidyl ether (BADGE)-based resins are predominantly used today.

BADGE is made from bisphenol A (BPA) and epichlorohydrin. The polymerisation of the resin takes place at higher temperatures and in the presence of various additional reactants (hardeners, cross-linkers, chain-stoppers, etc.) [2]. Remnants of the polymerisation, like BPA and BADGE (Fig. 1), are potential leachables, which are able to migrate from the layer material into the drug product. Furthermore, products of BADGE with water (BADGE·H_2O, BADGE·$2H_2O$) and chloride (BADGE·HCl, BADGE·2HCl and BADGE·HCl·H_2O) have to be considered as potential contaminants, as well.

Epoxy resins are, among other things, used as protective coating for foodstuff packaging (e.g. canned food and beverages). Studies verifying, quantifying and assessing migration of BPA from layer materials into food are reviewed by Kang et al. [3]. Furthermore, leaching of BADGE and its derivatives from can coatings and their determination in food was the object of several publications [4–6]. Based on the assessment of numerous toxicity, toxicokinetic and exposure studies [7,8] the use of BPA and BADGE for plastic materials and articles intended to come into contact with food is regulated by law [1,9].

Only few publications deal with leaching of BPA and BADGE from pharmaceutical packaging and medical devices. Migration of BPA from PVC bags into drugs [10] and from dialysis tubes into blood solutions [11] was monitored. Recently Søeborg et al. [12] developed an analytical method based on LC–ESI–MS–MS to determine bisphenol glycidyl ethers in a modified formula of *Aqueous Cream* of the British Pharmacopoeia. The cream was stored in one type of coated aluminium tube and migration was monitored under intensified conditions (70 °C, 120 h). The extent of migration was significantly lower when compared to the results after extraction of empty tubes with isopropanol for 48 hours at 70 °C.

Semi-solid dosage forms are typically used for topical applications on intact or damaged dermis and mucosa including ophthalmic and nasal application. Toxicological effects caused by BPA and BADGE after topical application are unclear due to lack of data which was already discussed by Søeborg et al. [12]. However, leachables from the container closure system are contaminants

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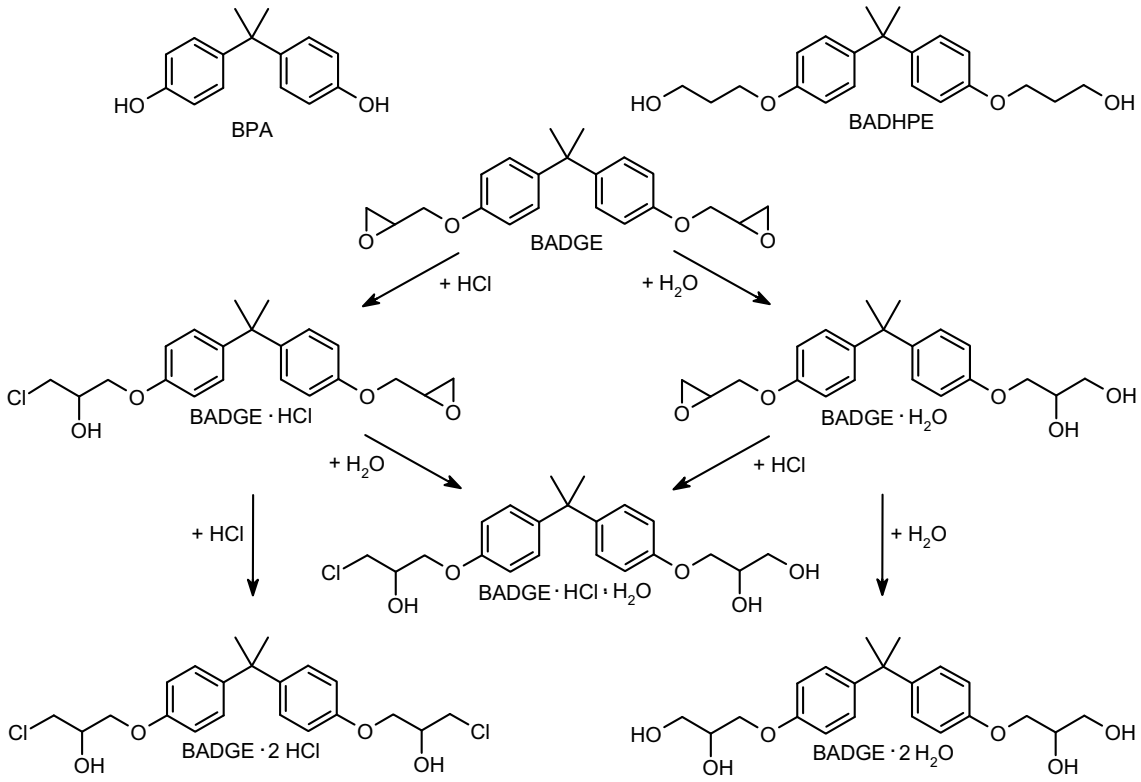

Fig. 1. Structures of bisphenol A (BPA), BADGE, its products with water and chloride, and the internal standard (BADHPE).

of the formulation. Notwithstanding a potential toxicological effect, contamination is always assessed as a negative impact on drug quality, which is among safety and efficacy a prerequisite on medicinal products. Additionally, the Federal Institute for Risk Assessment categorised BADGE to be an important allergen causing severe contact dermatitis [13]. In view of their topical administration, this is a further aspect why contamination of semi-solid dosage forms with BADGE should be prevented.

The objective of this study was to evaluate the maximum extractable amount of the aforementioned compounds from coatings of various types of aluminium tubes for pharmaceutical use and to monitor their leaching into different kinds of semi-solid dosage forms. Migration increasing factors should be evaluated.

2. Materials

2.1. Reference substances

Bisphenol A (BPA, ⩾99%, CAS No. [80-05-7]) and bisphenol A di-3-hydroxypropylether (BADHPE, CAS No. [37353-75-6]) were obtained from Sigma–Aldrich (Steinheim, Germany).

Standards of bisphenol A diglycidyl ether (BADGE, ⩾97%, CAS No. [1675-54-3]), bisphenol A (2,3-dihydroxypropyl) glycidyl ether (BADGE·H_2O, ≈97%, CAS No. [76002-91-0]), bisphenol A bis(2,3-dihydroxypropyl) ether (BADGE·2H_2O, ⩾97%, CAS No. [5581-32-8]), bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether (BADGE·HCl, ⩾90%, CAS No. [13836-48-1]), bisphenol A bis(3-chloro-2-hydroxypropyl) ether (BADGE·2HCl, ⩾97%, CAS No. [4809-35-2]) and bisphenol A (3-chloro-2-hydroxypropyl) (2,3-dihydroxypropyl) ether (BADGE·HCl·H_2O, ⩾95%, CAS No. [227947-06-0]) were purchased from Fluka (Buchs, Switzerland).

Stock solutions of all compounds were prepared in acetonitrile (1000 mg/L) and stored at - 20 °C. No degradation was observed during 12 months storage (results not shown). Prior to use, particular working standard solutions (1000 μg/L) were prepared in aliquots.

2.2. Chemicals

Acetonitrile was HPLC gradient grade (Sigma–Aldrich). Unless otherwise mentioned, further reagents were of analytical grade. *n*-Heptane and methanol were obtained from Sigma–Aldrich (Steinheim, Germany). Ammonium formate, formic acid, 1,2-propanediol and sorbic acid were purchased from Fluka (Buchs, Switzerland), acetic acid, acetone, ethanol, 2-propanol, sodium hydroxide and sodium sulphate anhydrous from Merck (Darmstadt, Germany). Refined olive oil, pure, was purchased from Acros Organics (Morris Plains, NJ, USA). Water was obtained from a Milli-Q water purification system (Millipore, Molsheim, France). Solid phase extraction was carried out on Bakerbond C18 500 mg/3 mL columns (Mallinckrodt Baker, Deventer, Holland).

All further matrix ingredients including the preservative potassium sorbate were of Ph.Eur. grade and purchased from Caelo (Hilden, Germany).

2.3. Matrices

About 1.5 kg *Einfache Augensalbe DAC* (eye ointment) [14], *Wasserhaltiges Carbomergel DAB* (water-based carbomer gel) [15], *Nichtionische Hydrophile Creme DAB* (nonionic hydrophilic cream), *Ultraschallkontaktgel NRF 13.2* (ultrasound gel) [16], *2-Propanolhaltiges Carbomergel DAB* (2-propanol-based carbomer gel) and ethanol-based carbomer gel were custom-made in a Unimix (Haagen & Rinau Mischtechnik, Bremen, Germany). The latter formula is proposed by the monograph of *2-Propanolhaltiges Carbomergel DAB*.

Water-based carbomer gel and nonionic hydrophilic cream were preserved according to the particular monograph.

2.4. Aluminium tubes

Seven kinds of tubes (4–10 mL) currently available for pharmaceutical packaging were purchased from seven different vendors. Two different batches of type A were obtained. All tubes were internally lacquered with epoxy resins.

2.5. Apparatus/HPLC methods

Analysis was based on RP-HPLC gradient elution coupled with fluorescence detection using a system from Dionex (Germering, Germany): P680A dual low-pressure gradient pump, ASI-100 autosampler, RF2000 fluorescence detector, STH 585 column oven (Gynkotek, Germering, Germany). A Multospher 100 RP18-5 μ, 250 × 4 mm column (CS-Chromatographie, Langerwehe, Germany) was used as stationary phase. The analytical method was taken over from Petersen et al. [17]. For the analysis of cream, the gradient was slightly flattened to enable sufficient separation. Furthermore, the chromatograms of the cream had to be manually evaluated after arithmetical background subtraction of matrix samples. Where necessary, the injection volume was altered between 10 and 100 μL to fit the validated working range. Samples were kept at 4 °C in the autosampler for a maximum of 48 h to prevent hydrolysis and were analysed in duplicate.

Semi-preparative purification was carried out on a HPLC system from Gynkotek: M 480 and M 300 CS pump combination, Gina 50 autosampler, STH 585 column oven, UVD 340 S detector (Dionex). A Jupiter 5 μ C18 300 Å 250 × 10.00 mm (Phenomenex, Aschaffenburg, Germany) was used as stationary phase. Isocratic elution was performed with methanol/water (60:40 V/V) at a flow rate of 3.8 mL/min (T = 30 °C).

3. Methods

3.1. Extraction studies

Empty aluminium tubes (type A) were extracted with various extraction media (water, acetic acid 3%, olive oil, methanol, ethanol, isopropanol, acetonitrile, and acetone) at 40 °C for 10 days. The nominal volume (5 mL) of the particular solvent was filled into tubes (n = 5) which were subsequently closed by manual folding. All tubes were fixed in a test tube rack with closures down. The rack was stored in a VC 0033 climatic test chamber (Vötsch Industrietechnik, Balingen-Frommern, Germany). Leak-tightness defined as <0.3% weight loss was controlled via differential weighing at the beginning and end of storage.

Except acetonitrile, water, and olive oil extracts, an aliquot (4 mL) of the content was evaporated to dryness (40 °C) with a Büchi 471 rotary evaporator (Büchi Labortechnik, Flawil, Switzerland). Afterwards the samples were redissolved in 4.2 mL acetonitrile and diluted with water to the final volume of 10 mL. Acetonitrile and water extracts were adjusted to reach the same solvent ratio. Aliquots of all solutions were filtered via 2 mL syringe (B.Braun Melsungen, Melsungen, Germany) and Chromafil PET-45/15 MS (Macherey-Nagel, Dueren, Germany). Olive oil (4 mL) was prepared in analogy with eye ointment (Section 3.2.2). Where necessary, the initial aliquot volume was reduced to fit the validated working range.

For another comparative tube extraction study, the particular nominal volume of acetonitrile (4–10 mL) was filled into tubes of seven different vendors (n = 5). Both batches of type A were taken into account. Storage and preparation were carried out in analogy with the previous description (40 °C, 10 days).

3.2. Migration study

Tubes of one supplier (type A) were manually filled with matrices, closed by folding and stored with closures down in a test tube rack under intermediate and accelerated conditions according to the current regulatory guideline for stability studies with drug products [18]. To monitor unspecific temperature effects, the matrix samples were simultaneously stored in glass containers. Sampling of tubes and blank matrices was done at intervals. Leak-tightness was controlled after sampling via differential weighing. In addition, cream samples were mechanically stressed by squeezing the tubes once a week to investigate the influence of in-use conditions. To exclude effects of batch-to-batch-variability, the usage of only one batch per each experimental series had been paid attention.

Prior to HPLC analysis quantitative extraction of the analytes from the semi-solid formulae was necessary. During the first migration study with eye ointment, water-based carbomer gel and nonionic hydrophilic cream the content of five tubes was homogenised. For the comparative carbomer gel study and the in-use study, sampling was varied using three separate homogenates of two tubes each.

Aliquots of 5.0 g matrix were weighed in flasks in triplicate and spiked with 1 mL acetonitrile containing 5.0 mg/L internal standard (BADHPE), giving a concentration of 1.0 mg/kg. For the ethanol- and isopropanol-based carbomer gel, the internal standard level had to be adjusted to higher levels in order to compensate baseline interferences. Considering the particular properties of the selected formulae, all samples were prepared according to their individually elaborated preparation scheme.

3.2.1. Carbomer gels

Nine millilitres acetonitrile and 1.3 g ammonium formate were added to the flask and the resulting suspension was transferred to a test tube. After precipitation of carbomer and separation of the aqueous phase, the upper acetonitrile layer was completely transferred and evaporated to dryness at 40 °C. The residue was redissolved in 2 × 0.9 mL MeOH/H_2O (65:35 V/V), filled to the final volume of 2 mL with same solvent and filtered subsequently. Where necessary, sample was further diluted with same solvent prior to injection.

3.2.2. Eye ointment

Twenty millilitres n-heptane and 9 mL acetonitrile were added to the flask and the matrix was suspended via vigorous shaking. In case of incomplete suspension, the sample was treated by ultrasound bath (Sonorex RK 100 H, Bandelin, Berlin, Germany) for a maximum of 5 min. Liquid–liquid-extraction was carried out using 2 × 5 mL acetonitrile. The combined acetonitrile fractions were evaporated to dryness (40 °C). Remaining lipoids were subsequently removed by inverse solid phase extraction. For this, the sample was transferred with 3 × 2 mL ACN/H_2O (70:30 V/V) on an SPE column, which had previously been activated with 2 mL acetonitrile and conditioned with 1 mL ACN/H_2O (70:30 V/V). The combined eluates were filled up to 10 mL with water.

3.2.3. Cream

Twenty millilitres n-heptane and 9 mL acetonitrile were added to the flasks and the matrix was suspended in analogy with eye ointment. 0.7 g ammonium formate was added before liquid–liquid-extraction leading to a three-layer-system, which enabled n-heptane and most of the water layer to be discarded in one step. Furthermore, to prevent foaming during evaporation it was necessary to freeze out ($\geqslant$2 h, - 20 °C) most of the cetostearyl alcohol, which was subsequently separated by filtration (595 1/2) folded filters, Ø 90 mm, Schleicher & Schuell, Dassel, Germany) over

sodium sulphate anhydrous. Inverse solid phase extraction was carried out in analogy with eye ointment.

3.3. Method validation

Linearity was evaluated by injecting 100 μL of standard mixtures containing all analytes at concentration levels of 50–500 μg/L (gradually raised by 50 μg/L) in triplicate. In a parallel trial 10–100 μL (gradually raised by 10 μL) of three standard mixtures ($c \approx 500$ μg/L) were injected to verify unaffectedness of linearity by injection volume. Appearance of individual residual plots was assessed and coefficients of determination (R^2) were calculated.

Recovery of the method was determined at three spiking levels: 20, 100 and 200 μg/kg ($n = 3$ for each level) for the eye ointment and the nonionic hydrophilic cream. The optimised sample preparation of the carbomer gel study enabled levels of 4, 20 and 40 μg/kg.

LOD and LOQ were calculated on the basis of the recovery data according to DIN 32645 [19]. Precision was likewise calculated from the recovery data for each spiking level.

Statistic calculations were performed in Microsoft® Excel 2007 (Microsoft Corporation, Redmond, USA).

3.4. Purification of BADHPE

Prior to application BADHPE was purified via semi-preparative HPLC. After injections of 1000 μL BADHPE (50 mg/L) in methanol/water (50:50 V/V), the fractions between 16.5 and 31 min were collected, combined and evaporated to dryness at 50 °C.

4. Results and discussion

4.1. Method validation

A chromatogram of a standard mixture is given in Fig. 2. All substances including the internal standard BADPHE are sufficiently separated by the selected method. Despite previous purification BADPHE resulted in two peaks: a main peak and a lower pre-peak. The peak area ratio between main and pre-peak remained constant during studies, thus, no degradation of BADHPE is assumed. Since no interference with other standard peaks became evident the impurity was disregarded.

Linearity was demonstrated for the working range between LOD and 500 μg/L. With regard to residual plots neither trends nor further peculiarity became obvious. All coefficients of determination exceeded 0.999.

The estimation of LOD and LOQ via signal-to-noise ratios of 3 and 10, respectively [as recommended by [20]], was precluded by interfering matrix-based peaks. For that reason LOD and LOQ had to be calculated on the basis of recovery data according to DIN 32645 [19]. Sufficient recoveries with acceptable precision were obtained and are presented in Table 1 as well as the results of the LOD and LOQ estimation. While recovery is concentration independent, precision is influenced in the expected way. Where possible, S/N-calculation was additionally carried out resulting in LODs and LOQs of comparable magnitude (not shown).

Furthermore, method robustness was verified with regard to, e.g. column oven temperature, buffer concentration, buffer pH, flow, etc. In MeOH/H_2O-mixtures used for the preparation of carbomer gels (Section 3.2.1), BADGE turned out to be less stable than in ACN/H_2O-mixtures. Since application of ACN/H_2O-mixtures is unsuitable due to phase separation after SPE caused by ammonium formate carry-over, use of MeOH/H_2O-mixtures was maintained. However, rapid sample preparation and sample cooling are mandatory for carbomer gels.

In conclusion, the presented validation data confirm suitability of the elaborated preparation scheme to quantify BPA, BADGE and its derivatives in the selected formulae with sufficient sensitivity, accuracy and precision.

4.2. Extraction study

Extraction studies were intended to evaluate whether free BPA, BADGE and its derivatives occur in tube coatings and to investigate to which extent they are extractable. To determine worst case conditions, a study with various media was carried out using empty aluminium tubes of one batch which was known for high analyte levels from previous studies (type A, batch #2). Extraction tests with volatile media were conducted with methanol, ethanol, isopropanol, acetonitrile and acetone. Furthermore, according to foodstuff legislation, water, acetic acid (3%), and olive oil were taken into account [21].

Among these different extraction media, acetonitrile and acetone provided the highest amounts of extractables under chosen

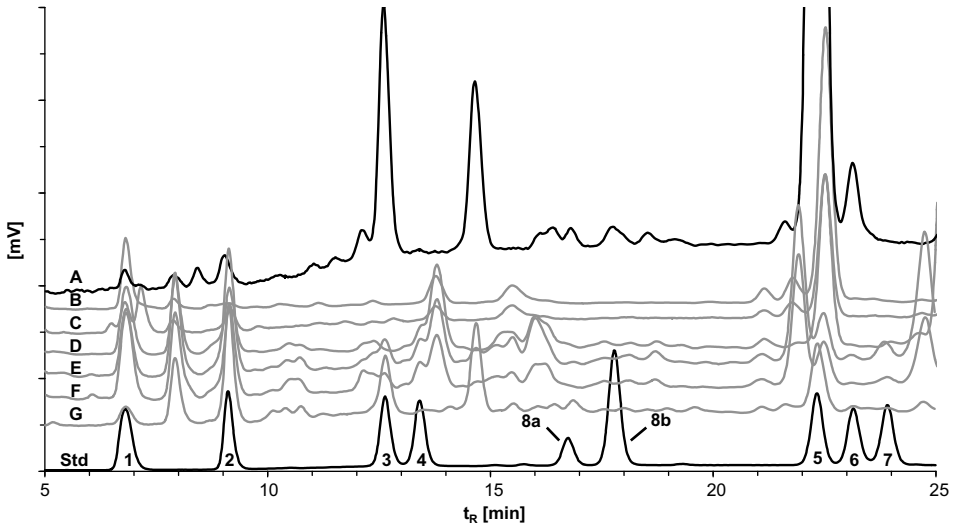

Fig. 2. Chromatogram excerpt of tube extracts vs. standard mixture ($c \approx 200$ μg/L). For the sake of clarity several chromatograms are compressed: extracts A (1:10), B (1:10), C (1:4) and D (1:1). 1, BADGE·2H_2O; 2, BPA; 3, BADGE·H_2O; 4, BADGE·HCl·H_2O; 5, BADGE; 6, BADGE·HCl; 7, BADGE·2HCl; 8a, Impurity of BADHPE; 8b, BADHPE ($c \approx 400$ μg/L).

Table 1
Characteristic validation data of different matrices spiked with 20, 100, 200 μg/kg ($n = 3$) or 4, 20, 40 μg/kg for the carbomer gels ($n = 3$), respectively

Analytical parameter	Compound						
	BADGE·2H$_2$O	BPA	BADGE·H$_2$O	BADGE·HCl·H$_2$O	BADGE	BADGE·HCl	BADGE·2HCl
Eye ointment							
LOD/LOQ	9.0/26.0 (∗)	2.8/8.2 (∗)	3.8/11.1 (∗)	9.5/27.3 (∗)	1.5/4.5	1.4/4.0	2.9/8.7 (∗)
Recovery	92.2/94.4/103.5	94.7/96.8/97.3	94.7/92.5/94.1	89.7/92.1/99.3	102.6/100.7/101.3	101.2/100.3/100.9	86.8/97.5/98.1
Precision	1.7–4.8	1.1–2.5	1.2–3.8	4.2–4.7	0.4–1.3	0.4–1.3	0.9–2.8
Nonionic hydrophilic cream							
LOD/LOQ	2.7/7.9	2.3/6.8	1.2/3.4	1.3/4.0	2.0/6.0	1.3/3.9	2.4/7.1
Recovery	99.5/98.9/100.0	101.7/100.9/100.2	99.9/99.7/99.6	99.9/100.5/99.8	103.9/99.0/98.7	101.9/100.2/99.5	98.3/99.9/98.8
Precision	0.2–1.9	0.8–4.9	0.3–2.9	0.2–2.1	0.1–0.9	0.3–0.8	0.3–5.8
Water-based carbomer gel							
LOD/LOQ	0.16/0.47	0.23/0.70	0.36/1.08	0.21/0.63	0.26/0.79	0.17/0.52	0.15/0.44
Recovery	94.5/96.0/95.2	109.2/108.8/105.1	93.5/96.1/95.9	94.2/101.2/99.4	103.1/96.8/97.4	98.7/98.7/98.8	98.2/102.5/100.9
Precision	0.2–2.7	0.1–3.1	0.3–1.4	0.2–2.3	0.2–1.5	0.2–1.3	0.2–1.2
Ultrasound gel							
LOD/LOQ	0.29/0.85	0.33/0.99	0.48/1.41	0.35/1.05	0.41/1.22	0.29/0.86	0.26/0.78
Recovery	82.6/85.1/86.8	104.5/104.1/103.0	98.2/100.7/99.4	99.2/101.2/100.2	99.7/101.2/100.6	101.8/102.2/101.7	103.4/101.3/102.3
Precision	0.2–1.8	0.1–0.9	0.2–3.8	0.4–1.0	0.3–1.6	0.3–1.9	0.3–1.0
Ethanol-based carbomer gel							
LOD/LOQ	0.31/0.92	0.49/1.45	0.31/0.92	0.34/1.01	0.62/1.84	0.45/1.34	0.42/1.26
Recovery	99.0/94.3/93.5	106.6/107.2/106.2	95.2/95.0/95.1	96.2/98.9/99.0	90.4/90.7/91.5	94.2/95.5/96.2	99.4/100.5/100.3
Precision	0.4–2.9	0.2–1.6	0.2–2.3	0.2–0.6	0.1–2.6	0.2–2.0	0.5–1.5
Isopropanol-based carbomer gel							
LOD/LOQ	0.30/0.90	0.31/0.91	0.37/1.09	0.24/0.72	0.59/1.75	0.41/1.21	0.37/1.10
Recovery	95.6/94.6/94.9	110.8/109.5/109.9	91.3/91.6/90.9	98.7/99.4/99.1	85.2/85.7/85.8	93.5/92.7/93.2	100.9/100.3/99.8
Precision	0.5–1.5	0.3–1.3	0.4–2.4	0.1–1.0	0.4–3.4	0.1–2.4	0.3–2.0

Limits of detection (LOD) and limits of quantification (LOQ), given in μg/kg, were calculated according to DIN 32645 [19]. Recoveries at the different concentrations and range of precision (RSD) are given in percentage. (∗) Due to absence of relevant peaks during migration study heteroscedasticity of labelled analytes was ignored.

conditions (Fig. 3). Tests via attenuated total reflectance (ATR)-FTIR with acetonitrile treated tubes revealed no significant spectra differences before and after extraction (not shown). This suggests that no physical alteration of the coating occurred. Extraction via alcoholic media resulted in significantly lower BADGE levels. However, in chromatograms of methanolic extracts compared to other extracts additional peaks were observed. These peaks are assumed to be products of BADGE and methanol as it has already been described for lower alcohols by Schaefer and Simat [22]. Hence, the lower BADGE concentration in alcoholic media could be a result of ether formation. In the food simulants water and acetic acid 3% only BADGE·2H$_2$O could be detected just above LOQ, in olive oil none of the analytes was even detectable. Thus, these solvents are considered unsuitable to determine the amount of non-cross linked BPA, BADGE and its derivatives in tube coatings. Finally acetonitrile and acetone turned out to be the most suitable extraction media to estimate the maximum amounts of extractables from epoxy-based tube coatings. Acetonitrile was selected for further investigations.

Additional experiments with acetonitrile revealed no further increase of the extractable amount after 10 days storage at 40 °C (data not shown). Consequently these conditions were defined to be suitable for further extraction studies, corresponding to the conditions required by food legislation [21].

In order to study extraction variability of tubes of different suppliers, the extraction profiles of seven tubes of different origin were compared. For a worst case examination small tubes were used to obtain high surface-to-volume ratios. Since closure extraction revealed no interference with analyte peaks, filled tubes were stored with closures down.

As demonstrated in Fig. 2, the extraction profiles revealed a plain variability in the nature and amount of extractables among

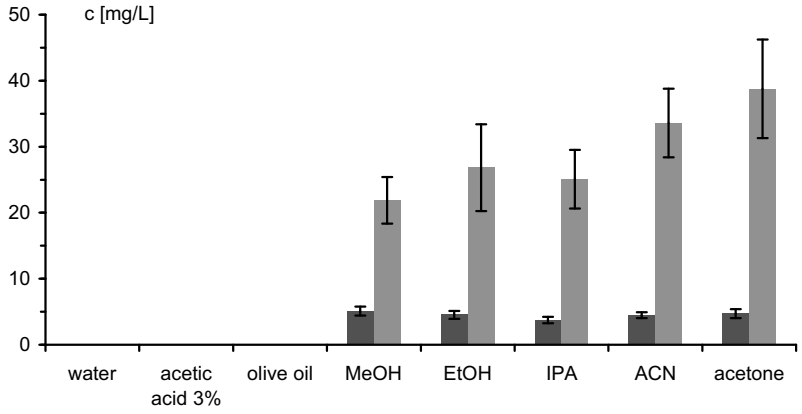

Fig. 3. Amounts of BADGE (light grey) and BADGE·H$_2$O (dark grey) extractable from aluminium tubes (type A, batch #2) with 5 mL of various media (MeOH, methanol; EtOH, ethanol; IPA, isopropyl alcohol; ACN, acetonitrile) stored for 10 days at 40 °C. Results are given with standard deviation ($n = 5$).

tubes of different vendors. Beside known analytes many other fluorescent extractables became visible, which might be reaction products of BADGE with further components of the lacquer as described by Schaefer and Simat [22]. Although certain resemblance between particular extractable profiles can be observed, specific peaks plainly differ.

BADGE·2H$_2$O was extractable from all coatings, whereas BPA, BADGE·H$_2$O, BADGE·HCl·H$_2$O, BADGE and BADGE·HCl were only detectable in certain extracts. Compared with tubes B-G extract A (batch #1) revealed 30- to 400-times higher concentrations of BADGE·H$_2$O and BADGE (Table 2), respectively. For this batch surface-related calculation of the specific migration of BADGE and the hydrolysed derivatives (BADGE·H$_2$O and BADGE·2H$_2$O) resulted under the chosen conditions in 11.7 mg/6 dm^2. With regard to the type of tubes Søeborg et al. [12] investigated, tubes of type B–D revealed levels of comparable magnitude, keeping in mind the different extraction conditions (2 mL isopropanol, 48 h, 70 °C). From the remaining tubes E–G the monitored compounds were merely extractable at significantly lower levels.

When a second batch of tubes type A was studied the monitored compounds were extractable at high levels, too, although clear differences to batch #1 existed (Table 2). This finding confirms not only the previous extraction results but also indicates significant batch-to-batch variability. As the composition of the epoxy resin was not changed by the vendor (personal communication and certificate of analysis) a crucial impact of the coating process to the amount of extractables must be assumed.

4.3. Migration studies

The aim of migration studies was to investigate whether contamination of semi-solid dosage forms by BPA and BADGE derivatives occurs during storage of medicinal products in aluminium tubes and to evaluate migration influencing factors. Only established formulae with well-described properties were considered, which also had to be microbiologically stable for at least 12 months. In order to simplify sample preparation and analysis only formulae with a few and, if available, pure components were taken into account. In order to cover the spectra of semi-solid dosage forms, three completely different kinds of representative matrices were selected: the lipophilic eye ointment, the hydrophilic water-based carbomer gel and nonionic hydrophilic cream. The influence of polarity on the rate migration was investigated in a second study using matrices of comparable compositions with assumed higher extraction potentials. For that reason three further carbomer gels were selected: ultrasound gel, 2-propanol-based carbomer gel and ethanol-based carbomer gel. Tubes of type A were used for all migration studies.

The results of the first migration study are shown in Fig. 4. Due to the selected sampling procedure, depicted intervals reflect method precision. The study revealed leaching in all matrices during storage at accelerated (40 °C) as well as intermediate conditions (30 °C). Among the derivatives studied hydrolysed BADGE·2H$_2$O and BADGE could be predominantly quantified. In cream additional leaching of BADGE·HCl·H$_2$O was observed at low levels after 6 months of storage at 40 °C (not shown). Comparing the outcome at both storage temperatures, intermediate conditions generally resulted as expected in analogue migration behaviour at significantly lower levels.

Apart from the expected correlation between migration and storage temperature, a distinct effect of the matrix on the extent of migration and on the leachable profile became evident. The highest amount of contamination in this study was observed in cream. For the study under accelerated conditions, the maximum extent of contamination seems to be reached after 6 weeks by asymptotic expansion. From that point on the sum of contaminants did not change significantly, but steady hydrolysis of BADGE to BADGE·2H$_2$O was monitored. The same effect could be observed with intermediate storage conditions at lower levels. However, when stored at 30 °C hydrolysis was not completed after 52 weeks of storage (Fig. 4), thus demonstrating the temperature dependence of hydrolysis kinetic, as expected. Equally the measured amount of BADGE·2H$_2$O in the water-based carbomer gel is supposed to be a product of BADGE hydrolysis during storage.

Migration of BADGE compounds from epoxy-based tube coatings into cream was also observed by Søeborg et al. [12], who reported BADGE·2H$_2$O, BADGE·H$_2$O, BADGE·HCl·H$_2$O and BADGE to be found in aqueous cream after storage at intensified conditions (70 °C). In their study the main peak BADGE·2H$_2$O reached a concentration of about 160 μg/L after 120 h of storage. These results are essentially confirmed by our examination: at the end of the storage, BADGE·2H$_2$O turned out to be the predominant leachable in cream, too. However, when monitoring the compounds over time, it became obvious that BADGE primarily leaches from the coating into the matrix, whereas BADGE·2H$_2$O is mainly the hydrolysis product of BADGE after leaching.

With regard to the results of the extraction study this first study revealed significantly lower levels of leachables (approx. 400–500-times lower).

In order to study the effect of matrix polarity on migration, different carbomer gels were stored in aluminium tubes at 30 and 40 °C for 6 months (Table 3). When compared to the leachables quantified in water-based carbomer gel, further derivatives could be detected especially in the alcohol-based carbomer gels. The highest amount of leachables was found in isopropanol-based carbomer gel, while the ultrasound gel was most similar in profile to water-based carbomer gel. In alcohol-based matrices hydrolysis after migration again became evident: the amounts of BADGE·2H$_2$O and BADGE·HCl·H$_2$O continuously increased while BADGE, BADGE·HCl and BADGE·H$_2$O (as intermediate) decreased (Fig. 1). The sum of BADGE and the hydrolysed products remained fairly constant even after 3 weeks of storage at both temperatures. The slightly different molecular weight did not alter this result significantly. This means that leaching from the coating into the matrix occurred mainly within the first 3 weeks.

Table 2
Results of the extraction study (40 °C, 10 days) with the nominal volume (4–10 mL) of acetonitrile

Tube type	BADGE·2H$_2$O	BPA	BADGE·H$_2$O	BADGE·HCl·H$_2$O	BADGE	BADGE·HCl	BADGE·2HCl
A #1	346 ± 38	748 ± 95	6260 ± 610	55.4 ± 8.7	57400 ± 4700	1580 ± 130	nd
A #2	168 ± 33	955 ± 104	4230 ± 230	56.9 ± 4.4	30900 ± 2000	1280 ± 80	nd
B	1480 ± 160	nd	nd	nd	nd	nd	nd
C	329 ± 172	nd	nd	nd	nd	nd	nd
D	858 ± 133	721 ± 53	nd	78.9 ± 9.7	nd	nd	nd
E	183 ± 10	339 ± 15	32.0 ± 2.7	30.8 ± 6.2	nd	nd	nd
F	247 ± 16	264 ± 14	18.1 ± 3.6	33.8 ± 6.0	nd	nd	nd
G	50.5 ± 8.4	307 ± 20	126 ± 40	nd	148 ± 63	nd	nd

Both batches of tube type A were considered. Average concentrations with standard deviations (n = 5) are given in μg/L. Assignment of peaks was confirmed via LC–MS against reference standards. nd = not detectable.

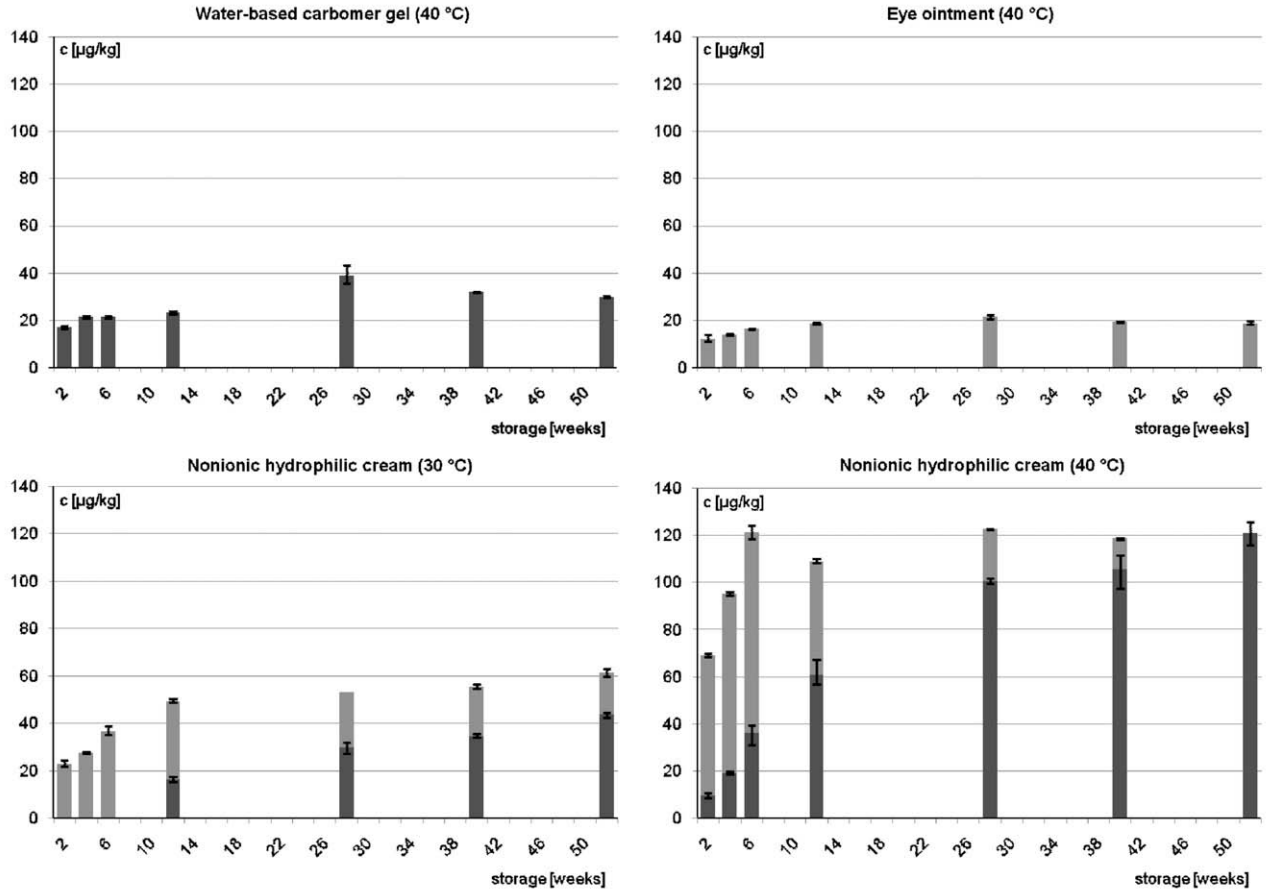

Fig. 4. Migration of BADGE (light grey) and BADGE·2H$_2$O (dark grey) into different matrices during storage (n = 3, tube type A, batch #1). Intervals reflect minimum and maximum amount measured. Results of intermediate storage conditions (30 °C) of eye ointment and water-based carbomer gel are omitted.

Table 3
Exemplary migration data of BPA, BADGE and its derivatives determined in three different carbomer gels during storage at 30 °C and 40 °C (tube type A, batch #2)

	Compound	Ultrasound gel			Ethanol-based carbomer gel			2-propanol-based carbomer gel		
		3 weeks	3 months	6 months	3 weeks	3 months	6 months	3 weeks	3 months	6 months
30 °C	BADGE·2H$_2$O	6.7	8.5	12.0	56.4	100	114	332	754	1260
	BPA	6.3	5.9	5.0	18.9	21.6	29.4	73.2	107	132
	BADGE·H$_2$O	2.1	nd	nd	52.6	5.7	3.4	752	105	29.6
	BADGE·HCl·H$_2$O	1.4	1.6	1.9	(∗)	(∗)	(∗)	24.8	54.4	78.5
	BADGE	1.9	nd	nd	22.9	2.4	<LOQ	541	27.3	2.9
	BADGE·HCl	nd	nd	nd	nd	nd	nd	42.7	7.8	nd
	BADGE·2HCl	nd	nd	nd	nd	nd	nd	nd	nd	nd
40 °C	BADGE·2H$_2$O	19.2	23.9	34.6	543	678	701	6710	8160	10800
	BPA	7.0	8.5	9.7	86.5	85.9	96.1	383	611	680
	BADGE·H$_2$O	1.5	nd	nd	86.2	nd	nd	3140	nd	nd
	BADGE·HCl·H$_2$O	2.2	2.9	3.9	(∗)	(∗)	(∗)	350	449	504
	BADGE	<LOQ	nd	nd	17.9	<LOQ	<LOQ	513	7.6	10.4
	BADGE·HCl	nd	nd	nd	nd	nd	nd	102	nd	nd
	BADGE·2HCl	nd	nd	nd	nd	nd	nd	nd	nd	nd

Average concentrations (n = 3) are given in µg/kg; (∗) Integration impossible due to overlapping peaks which arose. nd, not detectable.

Comparing the results of one gel at both temperatures, however, significantly higher levels of leachables were evident at 40 °C. As leaching was almost completed after 3 weeks of storage this fact cannot solely be explained by increased diffusion processes. It is assumed that temperature dependent physicochemical alteration of the matrix properties leads to an enhanced leaching of the compounds from the coating into the matrix.

Compared to the results of the worst case extraction levels of up to 30% of the sum of BADGE and the hydrolysed products were detected in isopropanol-based carbomer gel (40 °C).

4.4. In-use conditions

In the mechanically stressed tubes (type A, batch #2), additional peaks could be detected above LOQ (Table 4). When compared to cream in the same tubes, but in this case unstressed, the extent of migration was clearly increased 10-fold, which corresponds to about 8% of the worst case extraction. Whether or not this effect is caused by mechanical damage of the coating surface or the manually enhanced diffusion still has to be further investigated.

Table 4
Comparison of the migration of BPA, BADGE and its derivatives in stressed and non-stressed samples of nonionic hydrophilic cream during storage at 40 °C (tube type A, batch #2)

Compound	Non-stressed samples			"In-use"-conditions		
	6 weeks	3 months	6 months	6 weeks	3 months	6 months
BADGE·2H_2O	54.1	69.4	136	392	1010	1790
BPA	12.9	12.9	18.8	47.9	117	142
BADGE·H_2O	7.5	<LOQ	nd	110	93.6	43.7
BADGE·HCl·H_2O	<LOQ	4.3	7.7	19.3	55.2	92.1
BADGE	120	91.5	57.3	956	1100	725
BADGE·HCl	9.1	5.1	< LOQ	62.1	85.2	63.8
BADGE·2HCl	nd	nd	nd	nd	nd	nd

Average concentrations ($n = 3$) are given in μg/kg. nd, not detectable.

5. Conclusions

Leaching of BPA, BADGE and its derivatives into various semi-solid drug products during storage was verifiable. The extent of leaching was time- and temperature-dependent, as expected. Additionally, leachable profiles are decisively influenced by the composition of the matrix. In this context matrix polarity seems to play a crucial role. Another aspect to be considered is mechanical stress. In-use conditions, simulated by squeezing the tubes, strongly increased the migration process. Thus, general prediction of the magnitude of migration into drug products is difficult due to obvious multi-factorial effects.

Quality data of the investigated tubes were merely made available by the vendors. Only one certificate of analysis was provided and refers to migration testing according to food law. As demonstrated by migration studies leaching of BPA, BADGE and its derivatives into drug products during storage can significantly exceed the results of extraction tests with food simulants. Thus, these media are considered unsuitable for estimating the magnitude of migration from coatings of aluminium tubes for pharmaceutical use during storage.

The results of the studies demonstrate that the quality of semi-solid dosage forms can be compromised by use of containers of minor quality. Allergic reactions after topical application caused by BADGE-contaminated products are conceivable but still have to be investigated. In order to ensure quality and safety of semi-solid formulae interactions between the coating material and the drug product should be thoroughly evaluated. Hence, extraction testing of coated tubes using suitable media and conditions and taking into account all extractable compounds is considered necessary for the quality assessment of the final container closure system.

In the current studies, further potentially influencing factors on the rate of migration are being investigated.

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