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Adsorption of peptides at the sample drying step: Influence of solvent evaporation technique, vial material and solution additive

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ABSTRACT

Although the efficient and careful removal of solvent from samples by centrifugal evaporation or freeze-drying methods is an important step in peptidomics, the recovery of peptides has not yet been fully investigated with these sample drying methods. Moreover, the surface adsorption of the peptides by the container and efforts to reduce this adsorption by organic additives is only scarcely elaborated until now. In this experiment, the recovery of five model peptides, *i.e.* bovine insulin, mouse obestatin, goserelin, buserelin and leucine-enkephalin was investigated applying dimethylsulfoxide (DMSO), dimethylformamide (DMF), polyethylene glycol 400 (PEG 400), mannitol and *n*-nonyl-β-D-glucopyranoside (C_9-Glu) in function of the two applied solvent evaporation processes (freeze-drying vs. centrifugal evaporation) and vial types, *i.e.* polypropylene (PP) and glass. Under our experimental conditions, drying resulted in a decreased recovery of the model peptides by 10% on average. Insulin showed the lowest recovery value relative to the other model peptides. For both drying methods, recovery of the model peptides was increased when C_9-Glu was present. Overall, the use of PP vials is proposed for freeze-drying, while glass vials are found to be more suitable for centrifugal evaporation. The presence of PEG 400 in PP vials caused significantly reduced recoveries for all model peptides using centrifugal evaporation, although this was not observed in glass vials. As a general conclusion, applying C_9-Glu as an additive along with choosing appropriate vial type (*i.e.* PP for lyophilization and glass for centrifugal evaporation) can avoid or diminish peptide loss during the evaporation procedure.

1. Introduction

Variable recovery of peptides is a well-known but often neglected phenomenon that impacts quantitative peptide analysis [1]. The adsorption of peptides/proteins to solid surfaces is believed to be due to noncovalent interactions (*e.g.* electrostatic, hydrophobic) and depending upon the experimental conditions (*e.g.* peptide properties, physical state of the surface and sample environmental properties). Only recently, few studies have systematically investigated the factors influencing the adsorption of peptides, however, without including the drying step in the sample preparation [2–4]. Nevertheless, the sample concentrating and drying step can be a significant source of peptide loss, mainly due to adsorption.

The influence of five surfactants on the equilibrium adsorption of salmon calcitonin and bovine serum albumin on hydrophilic glass and hydrophobic polypropylene (PP) surfaces was previously studied [5]. The hydrophobic content of the surfactant was found to be the greatest determinant in reducing the peptide/protein adsorption. In their method development for the quantification of the 36-amino-acid (AA) peptide pentafuside, Lawless et al. [6] observed adsorption of the peptide to the vial wall, which they presumably assigned to hydrophobic interactions. Several detergents were tried, leading to the use of 1% *n*-nonyl-β-D-glucopyranoside (C_9-Glu) as the best solution [6]. Adsorption is not only an analytical issue, but can be a formulation challenge as well. The adsorption of the 32-AA peptide calcitonin on soda lime silica glass was investigated varying the concentration, temperature, pH, preservatives and surface-active additives [7]. No temperature effect was found between 4 °C and 37 °C, but a pH–concentration interaction was demonstrated, as well as a decreasing adsorption with the preservative chlorobutanol and non-ionic surfactants such as Pluronic F68 and Tween 80. Following initial observations of adsorption of the cyclic D-AA containing heptapeptide microcystin-LR to polyvinyl chloride (PVC) containers, the effect of solvent and disposable PP pipette tips were subsequently studied using HPLC-PDA: the peptide was found to adsorb to the tips with a loss of 4.2% from a 4 μg/ml

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aqueous solution per tip operation, independent of pH and salinity, but dependent on the concentration of methanol and acetonitrile [2,3]. After an initial sample preparation for the assay of calcitonin in serum, a freeze-drying step was included and a reconstitution solvent containing acidified acetonitrile and benzalkonium chloride (BC) was found to be optimal in the LC–MS quantification as BC significantly increased the peak area, however without any further investigation [4]. In their review about quantification of peptides, John et al. [1] devoted special attention to adsorption, explicitly stating the results of their own experience with an undisclosed model peptide: the addition of acetonitrile or of C_9-Glu, as well as its concentration, did influence the adsorption to PP vials [1]. In a recent study, the adsorption of the decapeptide cetrorelix was found to follow a Langmuir isotherm and was found to be influenced by both the solvent medium and the type of vial used [8]. The repeatability of HPLC-responses was investigated using cytochrome *C* tryptic digest, and the unsatisfactory results were found to be mainly due to poor recovery of peptides from the sample vial, which the authors largely solved by addition of an optimal quantity of the organic modifier dimethylsulfoxide (DMSO) [9]. The sample storage of BSA tryptic peptides in regular versus low-retention PP sample tubes was compared: significant quantitative and qualitative differences on peptide recovery were found [10].

The aim of the study described here was to investigate the adsorption of five physicochemically different but pharmaceutically relevant model peptides, *i.e.* insulin, obestatin, buserelin, goserelin and leucine-enkephalin (molecular weight ranging from 555 to 5734) in function of the two applied drying processes (lyophilization vs. centrifugal evaporation), two vial materials (glass vs. PP) and five non-protein additives: DMSO, dimethylformamide (DMF), polyethylene glycol 400 (PEG 400), mannitol and C_9-Glu by RP-HPLC-PDA quantification. The influence of the additives on the chromatographic behaviour of the model peptides without a drying step was first evaluated. The accuracy and variability in recovery of each of the peptides after the drying step as usually performed in peptide analysis were then investigated using a full experimental design encompassing all combinations of the factors.

2. Materials and methods

2.1. Materials

High-quality model peptides were purchased from different suppliers: buserelin and goserelin from EDQM (Strasbourg, France), bovine insulin and leucine-enkephalin from Fluka (Buchs, Switzerland), and mouse obestatin [11] from California Peptide Research (Napa, CA, USA). Physicochemical properties of the selected model peptides are given in Table 1. Model peptides were dissolved in a mixture of 5% (v/v) acetonitrile in water, containing 0.1% (w/v) trifluoroacetic acid, lyophilized in 100 μg aliquots, and stored at −35 °C until use. HPLC gradient grade acetonitrile was obtained from Fisher Scientific (Leicestershire, UK). LC–MS grade formic acid, DMF, DMSO, PEG 400 and C_9-Glu were obtained from Fluka (Buchs, Switzerland). Mannitol was purchased from CERTA (Braine-l'Alleud, Belgium). Water was purified using an Arium 611 purification system (Sartorius, Gottingen, Germany) yielding ≥18.2 MΩ cm quality water. Total recovery HPLC glass vials were purchased from Waters (Milford, MA, USA). PP vials were obtained from Eppendorf (LoBind quality; Hamburg, Germany).

2.2. Liquid chromatography

The HPLC-PDA apparatus consisted of a Waters Alliance 2695 separations module and a Waters 2996 photodiode array detector with Empower 2 software for data acquisition (all Waters, Milford, MA, USA). UV spectra were recorded at 190–400 nm, and quantification was done at 275 nm.

A Vydac Everest RP-C_{18} (250 mm × 4.6 mm I.D., 5 μm particle size, 300 Å) column (Grace Vydac, Hesperia, CA, USA) in an oven set at 30 °C, with a mobile phase consisting of (A) 0.1% (w/v) formic acid in water and (B) 0.1% (w/v) formic acid in acetonitrile was used in this experiment. The gradient program employed was isocratic for 1 min at 90% (v/v) A and 10% (v/v) B, followed by a linear gradient to 60% (v/v) A + 40% (v/v) B at 60 min. The flow rate was set at 1.0 ml/min.

2.3. Effect of additives on chromatographic behaviour

In order to exclude an influence of injection solvent composition (*i.e.* the presence of the additives) on the chromatographic behaviour and recovery of the model peptides, a mixture of model peptides (0.2 μg/μl each), prepared in a 95/5% (v/v) water/acetonitrile solvent, was pipetted into total recovery glass vials containing the five separate additives, *i.e.* DMSO, DMF, PEG 400, mannitol (0.5% (w/v) aqueous solution) and C_9-Glu (0.05% (w/v) aqueous solution). 95 μl of model peptides mixture was added to vials containing the 5 μl of selected additives (*i.e.* 5% (v/v) additive or additive solution was used). Without drying involved, the samples were analyzed by the above HPLC method for evaluation of recovery and chromatographic characteristics, and compared with a control solution containing no additives. Each HPLC sample was independently prepared in triplicate and then each sample injected once, *i.e.* one HPLC run/result obtained from each individual sample. The chromatographic characteristics were automatically calculated with Empower 2 according to Ph. Eur. formulae [12].

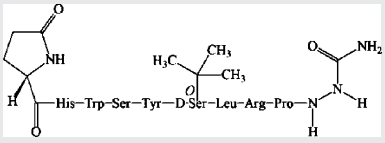
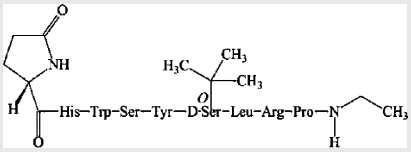
2.4. Residual DMSO and DMF after drying

A supplementary HPLC analysis was conducted to quantify the residual DMSO and DMF after drying (freeze drying and centrifugal evaporation, in both PP and glass vials). For this purpose, an Alltima RP-C_{18} (250 mm × 4.6 mm I.D., 5 μm particle size, 100 Å) column (Grace Vydac, Hesperia, CA, USA), kept at 30 °C, was used with a solution of 2.5% (v/v) of acetonitrile in water at 1 ml/min as mobile phase. Quantification was performed using UV detection at 220 nm. Using this isocratic system, typical retention times of 5.9 min and 10.8 min were obtained for DMSO and DMF, respectively. Test solutions were prepared ($n = 3$ for each additive and vial type) by drying 100 μl of 95/5% (v/v) water/additive solution using Lyovac and Speedvac under the conditions mentioned in Section 2.5, followed by the addition of pure water and sonication. Untreated (*i.e.* without drying) 95/5% (v/v) water/additive solutions were used as reference solutions ($n = 3$ for each additive).

2.5. Influence of peptide concentration

In order to investigate the effect of concentration on peptides adsorption, we conducted an experiment with our five model peptides. In this experiment, different concentrations of model peptides (200, 100, 20 and 2 μg/ml each) mixtures were prepared in ACN/water (5/95%, v/v). These model peptide mixtures were prepared in polypropylene vials and dried by Speedvac. The residue was redissolved in ACN/water (5/95%, v/v) and the resulting samples were analyzed by HPLC under conditions explained in Section 2.2.

Table 1
Some physicochemical characteristics of used model peptides.

Peptide	Chemical structure	Molecular formula	M_r	# AA	clog P[a]
Leucine-enkephalin	H—Tyr–Gly–Gly–Phe–Leu—OH	$C_{28}H_{37}N_5O_7$	555.6	5	−1.75
Mouse obestatin	H—Phe–Asn–Ala–Pro–Phe–Asp–Val–Gly–Ile–Lys–Leu–Ser— Gly–Ala–Gln–Tyr–Gln–Gln–His–Gly–Arg–Ala–Leu—NH_2	$C_{114}H_{174}N_{34}O_{31}$	2516.8	23	−17.34
Bovine insulin	H–Gly–Ile–Val–Glu–Gln–Cys–Cys–Ala–Ser–Val–Cys–Ser— Leu–Tyr–Gln–Leu–Glu–Asn–Tyr–Cys–Asn–OH H—Phe–Val–Asn–Gln–His–Leu–Cys–Gly–Ser–His–Leu–Val— Glu–Ala–Leu–Tyr–Leu–Val–Cys–Gly–Glu–Arg–Gly–Phe— Phe–Tyr–Thr–Pro–Lys–Ala–OH	$C_{254}H_{377}N_{65}O_{75}S_6$	5733.6	51	−30.65
Goserelin		$C_{59}H_{84}N_{18}O_{14}$	1269.4	9	−9.1
Buserelin		$C_{60}H_{86}N_{16}O_{13}$	1239.4	9	−7.86

[a] clog P was calculated by Hyperchem. 8.0.

2.6. Effect of additives on model peptide recovery including drying and vial material effects

For the assessment of peptide recovery after each of the two drying steps along with different vial materials and organic additives, a 0.2 μg/μl mixture of model peptides (*i.e.* bovine insulin, buserelin, goserelin, leucine-enkephalin, and mouse obestatin) was prepared in water. Five microliters of DMF, DMSO, PEG 400, water, mannitol solution (0.5% (w/v) in water) and C_9-Glu solution (0.05% (w/v) in water) were pipetted into both PP and total recovery glass vials containing 95 μl of the model peptides mixture. After drying the samples by centrifugal evaporation (Speedvac, Thermo Electron Corporation, MA, USA) or freeze-drying (Lyovac, Leybold, Cologne, Germany), 95 μl of water/acetonitrile mixture (95/5%, v/v) was added to the samples. Recovery values were corrected for evaporation of DMSO, DMF and water during drying. Each sample, independently prepared in triplicate, was transferred to total recovery HPLC glass vials and then each sample injected once, *i.e.* one HPLC run/result obtained from each individual sample ($n = 3$). The amounts of model peptides remaining in solution were quantified by the HPLC method described above. Temperature, vacuum pressure and run time for Speedvac were set at 45 °C, 5.1 Torr and 1 h, respectively. The program used for lyophilization with Lyovac is presented in Table 2.

2.7. Data treatment

The drying recovery, as presented in Table 3, is calculated from the without and with drying experiments as the ratio of the values. The PROC MIXED procedure of SAS software version 8.2 (SAS Institute Inc., Cary, NC, USA) [13] was used to analyze the data as a completely randomized design with repeated measurements. The models used to analyze the data included the fixed effects of evaporation technique, vial material, peptide, organic modifier, four-way interaction terms of fixed effects, and the residual errors. The significant difference level was set as $P < 0.05$.

Table 2
Lyovac program.

Process step	Time (min)	Shelve temperature (°C)	Chamber pressure (mbar)
1	0	20	1000
2	5	2	1000
3	25	−5	1000
4	85	−30	1000
5	145	−45	1000
6	165	−45	1000
7	175	−15	0.8–1.0
8	195	−15	0.8–1.0
9	975	−15	0.8–1.0
10	977	0	0.1–0.2
11	1097	10	0.1–0.2
12	1517	10	0.1–0.2

3. Results and discussion

Freeze-drying and centrifugal evaporation are commonly used as solvent evaporating methods in peptidomic studies. However, to our best knowledge, the peptide loss due to adsorption has not been studied during the drying step. For investigation of the overall effect of the drying step on the recovery of peptides under standard operational conditions, two independent experiments were conducted. In a first experiment, the sole influence of the different organic additives (*i.e.* without drying step) on recovery and chromatographic characteristics of the five model peptides was investigated (see Section 3.1). In a second experiment, the effect of the two different drying methods on the recovery of the model peptides was studied with the same additives used in the first experiment (see Section 3.2). The results of both experiments were compared to answer the key question whether the drying step may induce peptide loss during sample preparation. In our study, a generally applied peptide solvent was used for the sample reconstitution, *i.e.* a solution of 5% (v/v) acetonitrile in water. This solvent dissolves the investigated model peptides quite well, and is also compatible with the reversed phase HPLC used. Moreover, as the reconstitution proce-

Table 3
Effect of drying step on the recovery of individual model peptides in function of five additives and two vial materials (mean ± S.D.).

Peptide	Additive[a]	Drying recovery (%)			
		Speedvac		Lyovac	
		PP[b]	Glass	PP[b]	Glass
Leucine-enkephalin	Mannitol	90.3 ± 3.5	98.9 ± 4.2	89.0 ± 2.9	94.6 ± 3.4
	Water	97.6 ± 9.1	97.2 ± 8.3	93.2 ± 7.4	96.2 ± 9.1
	DMSO	96.5 ± 5.5	93.2 ± 4.7	85.1 ± 4.7	91.4 ± 3.4
	DMF	89.8 ± 3.5	101.9 ± 1.0	90.9 ± 1.6	93.3 ± 3.3
	PEG 400	89.4 ± 3.3	97.8 ± 3.6	83.4 ± 5.2	93.3 ± 3.1
	C_9-Glu	95.9 ± 1.6	100.9 ± 2.9	91.4 ± 2.0	95.0 ± 1.4
Goserelin	Mannitol	92.3 ± 4.6	92.3 ± 6.7	99.2 ± 3.1	64.3 ± 3.6
	Water	84.4 ± 4.5	102.2 ± 8.8	100.2 ± 4.7	73.5 ± 5.3
	DMSO	88.8 ± 5.1	100.6 ± 5.7	96.1 ± 5.5	72.1 ± 4.5
	DMF	89.7 ± 6.8	90.8 ± 7.2	92.4 ± 5.8	72.8 ± 6.5
	PEG 400	85.7 ± 8.2	92.8 ± 7.8	71.4 ± 6.6	54.0 ± 7.7
	C_9-Glu	93.1 ± 1.3	92.9 ± 8.3	90.4 ± 2.5	90.7 ± 2.5
Bovine insulin	Mannitol	62.8 ± 0.8	83.6 ± 4.2	81.7 ± 4.0	94.7 ± 5.3
	Water	82.4 ± 5.1	83.9 ± 6.0	90.3 ± 6.1	94.8 ± 2.4
	DMSO	92.6 ± 7.1	92.5 ± 7.4	94.1 ± 8.4	103.6 ± 9.2
	DMF	74.1 ± 2.7	89.2 ± 4.5	91.8 ± 5.1	98.6 ± 3.8
	PEG 400	95.8 ± 5.5	81.0 ± 5.2	64.7 ± 2.1	95.7 ± 3.3
	C_9-Glu	95.4 ± 7.1	92.1 ± 6.7	89.3 ± 5.0	88.9 ± 4.9
Buserelin	Mannitol	91.9 ± 4.4	91.5 ± 1.4	85.0 ± 3.1	71.9 ± 0.9
	Water	86.4 ± 3.8	91.6 ± 8.6	89.5 ± 1.5	82.4 ± 2.7
	DMSO	86.4 ± 1.8	93.4 ± 1.2	103.7 ± 2.1	81.7 ± 2.1
	DMF	91.1 ± 2.4	90.1 ± 1.2	94.2 ± 6.7	77.7 ± 0.9
	PEG 400	79.0 ± 2.2	105.7 ± 3.3	94.1 ± 2.4	79.2 ± 5.0
	C_9-Glu	96.2 ± 1.7	92.0 ± 3.8	92.0 ± 2.4	95.1 ± 1.7
Mouse obestatin	Mannitol	95.3 ± 3.1	93.7 ± 4.4	90.8 ± 6.0	95.2 ± 4.7
	Water	90.8 ± 2.8	91.1 ± 11.0	91.2 ± 3.0	88.5 ± 4.0
	DMSO	99.8 ± 3.7	91.2 ± 6.1	95.8 ± 4.0	91.1 ± 2.7
	DMF	101.4 ± 7.8	90.2 ± 7.4	101.3 ± 8.0	91.8 ± 7.0
	PEG 400	101.8 ± 8.3	93.2 ± 3.4	82.7 ± 4.4	87.2 ± 1.5
	C_9-Glu	97.2 ± 2.6	102.5 ± 3.6	92.6 ± 2.8	96.1 ± 2.3

[a] DMSO, dimethylsulfoxide; DMF, dimethylformamide; PEG 400, polyethylene glycol 400; C_9-Glu, *n*-nonyl-β-D-glucopyranoside.
[b] PP: polypropylene.

dure and solvent used in our study were kept identical for all dried samples, the relative influence of drying procedure, vial material, additive and peptide can be compared. However, it is clear that when the reconstitution conditions would be changed, *e.g.* solvent, temperature or time, other results can be obtained.

3.1. Recovery of model peptides without drying step

The recovery and chromatographic behaviour of the five model peptides (*i.e.* bovine insulin, mouse obestatin, buserelin, goserelin and leucine-enkephalin) were assessed applying five additives (*i.e.* DMSO, DMF, mannitol, PEG 400 and C_9-Glu) in the same vial material. For each peptide, the recovery obtained using water as solvent (*i.e.* without additive) was taken as a reference and hence set at 100%. In general, no statistically significant effect attributed to the presence of the additives on recovery was found for each model peptide. As expected, the chromatographic characteristics for each model peptide (*i.e.* plate count, symmetry factor and capacity factor) were not influenced significantly either by the presence of the organic additives. It is clear that the results of the without drying step indicate no significant in-solution adsorption of the peptides under our conditions.

3.2. Effect of drying technique on peptide recovery in function of additive and vial material

The influence of drying step on the recovery of individual model peptide as a function of additive and vial material is summarized in Table 3. Our results clearly demonstrate that peptides are lost during the drying step of the sample preparation: under our experimental conditions, the drying action resulted in an average decrease in model peptide recovery of 10% (average drying recovery = 90.3%, standard deviation = 4.46%, $n = 360$). Regardless of applied additives, model peptides and vial materials, a significant difference on peptide recovery was found between the two drying techniques (92.1 for Speedvac vs. 87.1% for Lyovac, respectively; $P = 0.0001$).

Since adsorption is a concentration dependent surface phenomenon, an additional experiment was conducted to investigate this concentration effect for the model peptides and container types used under the aforementioned conditions (see Section 2.5). Our results clearly confirmed the expected concentration effect (see Fig. 1), which can be adequately described by the Freundlich equation ($R^2 \geq 0.999$). Therefore, the adsorption effects observed in our study, obtained at relatively high concentrations, are most significant in the sense that they are expected to significantly increase at lower concentrations. From Fig. 1, it can be concluded that at lower concentrations, higher loss of peptides is observed due to adsorption to vial walls, consistent with previous literature findings [1].

The drying recovery of the model peptides as a function of the two drying techniques and vial materials is presented in Fig. 2. When the recovery of model peptides in PP and glass vials was compared between Speedvac and Lyovac, the following results were obtained: (1) higher recovery of model peptides in glass vials than PP vials using Speedvac (93.8% for glass vs. 90.5% for PP; $P = 0.0001$); (2) less recovery of model peptides in glass than PP using Lyovac (84.2% for glass vs. 90.5% for PP; $P = 0.0001$). Our data clearly showed that the used vial materials significantly and differently influenced the recovery of model peptides when using Speedvac and Lyovac drying methods. In terms of preventing peptide loss during drying,

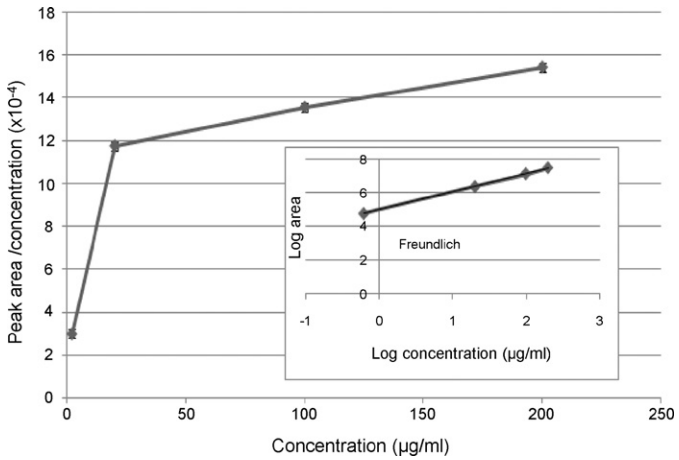


Fig. 1. Influence of different model peptides concentrations (200, 100, 20 and 2 µg/ml) on adsorption to polypropylene container surfaces. Each point on the graph represents the mean of the calculated peak area/concentration for the five selected model peptides.

PP vials are more suitable for lyophilization, while glass vials are proposed for centrifugal evaporation.

The influence of the two drying techniques on the recovery of model peptides in function of organic additives and vial materials is shown in Fig. 3. Although the recovery of freeze-dried model peptides was statistically lower in glass vials than in PP vials, no significant difference was found between the two vial types in combination with PEG 400 and C_9-Glu (Fig. 3B). However, for the centrifugal evaporated model peptides in the presence of PEG 400, the recovery was found to be statistically higher for glass tubes versus PP: 95.0% and 90.3%, respectively. PEG 400 seems to promote peptide adsorption onto PP surfaces. This phenomenon can be explained as an increase of hydrophobic interactions between the peptides and the PP surface due to an increase of the hydrophilicity of the medium. In general, using Speedvac as a drying technique showed more consistent results relative to the additive, *i.e.* this drying technique was more robust and less influenced by the additive presence.

The overall recovery values of model peptides when different organic additives were applied ranged from 86% to almost 94% (*i.e.* when mannitol and C_9-Glu is used, resp.). From the above results, it is apparent that under the conditions used, C_9-Glu is the best additive for recovery of peptides during centrifugal evaporation as well as during freeze-drying: this additive not only showed the highest recovery, but also gave more robust results, *i.e.* less influenced by

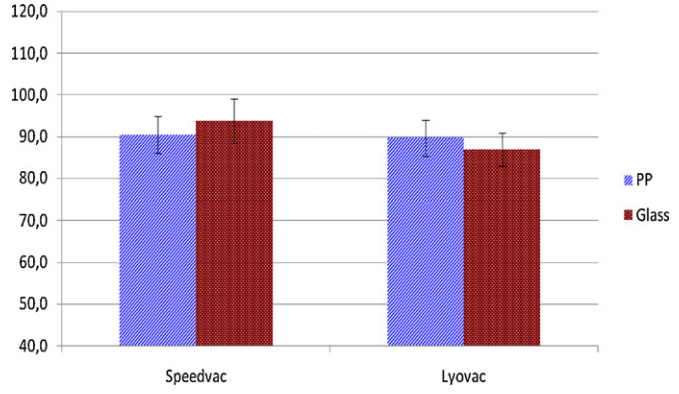


Fig. 2. Influence of drying methods (*i.e.* Speedvac and Lyovac) on drying recovery (%) of the model peptides in function of two different container materials, *i.e.* glass and polypropylene (PP) (mean bar plots ± S.E.M.).

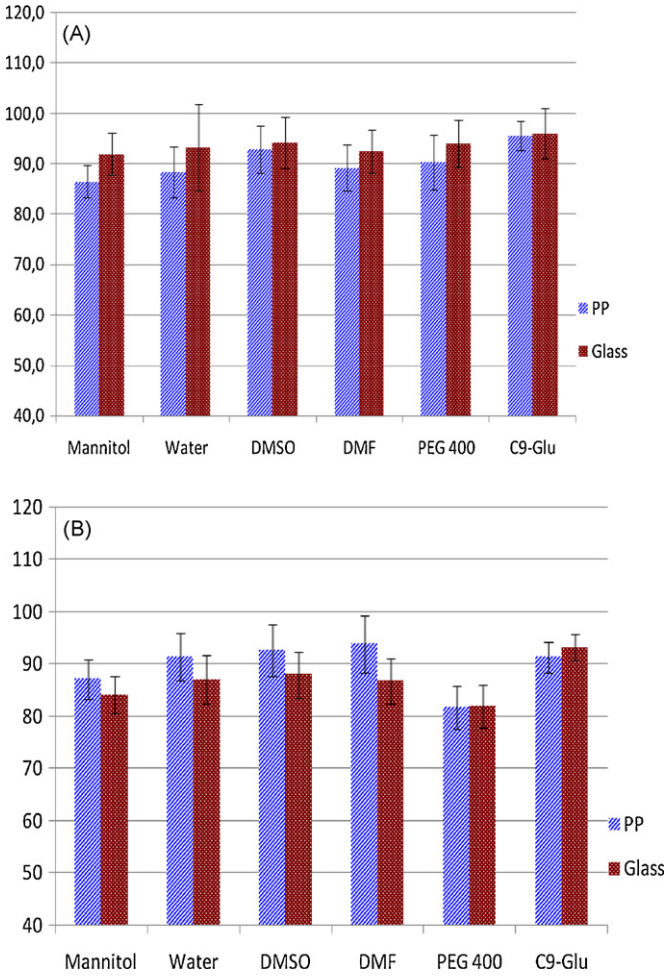


Fig. 3. Influence of additives (*i.e.* mannitol, water, DMSO, DMF, PEG 400 and C_9-Glu) on drying recovery (%) of the model peptides in polypropylene (PP) and glass vials using Speedvac (A) and Lyovac (B) (mean bar plots ± S.E.M.).

the operational variables. Lawless et al. [6] demonstrated that the application of 1% C_9-Glu in acetonitrile extraction mixture can avoid adsorption of pentafuside to PTFE and/or glass sample container surfaces. John et al. [1] showed that the addition of acetonitrile or C_9-Glu can reduce the adsorption of an undisclosed model peptide to PP vial walls. Especially when applying lyophilization, a diminished adsorption of model peptides was observed for DMSO, DMF and water as well (see Fig. 3B). Borges et al. [14] and Szabo et al. [15] reported improved results of relative standard deviation for direct-infusion ESI-MS of hydrophobic compounds (including synthetic hydrophobic peptides) when adding DMSO. This solvent is also used for solubilization of peptides and proteins in fundamental biological and biophysical studies [16,17], and has the unique property that it efficiently dissolves both polar and apolar compounds. DMSO can make hydrogen bindings with water molecules, while at the same time efficiently solvating hydrophobic parts of the peptide, thus creating a favorable network with water for dissolving the peptide. From a practical point of view, we demonstrated that DMSO evaporated completely after drying by Lyovac in both vial materials, while the recovery of DMSO after drying by Speedvac was 17.3% and 7.9% in PP and glass vials, respectively. In case of DMF, it was completely evaporated after drying by Speedvac and Lyovac in both PP and glass vials.

The influence of the peptide on drying recovery in the two materials, *i.e.* glass and PP, using Speedvac and Lyovac is depicted in Fig. 4. Regardless of other factors (*i.e.* drying, vial materials

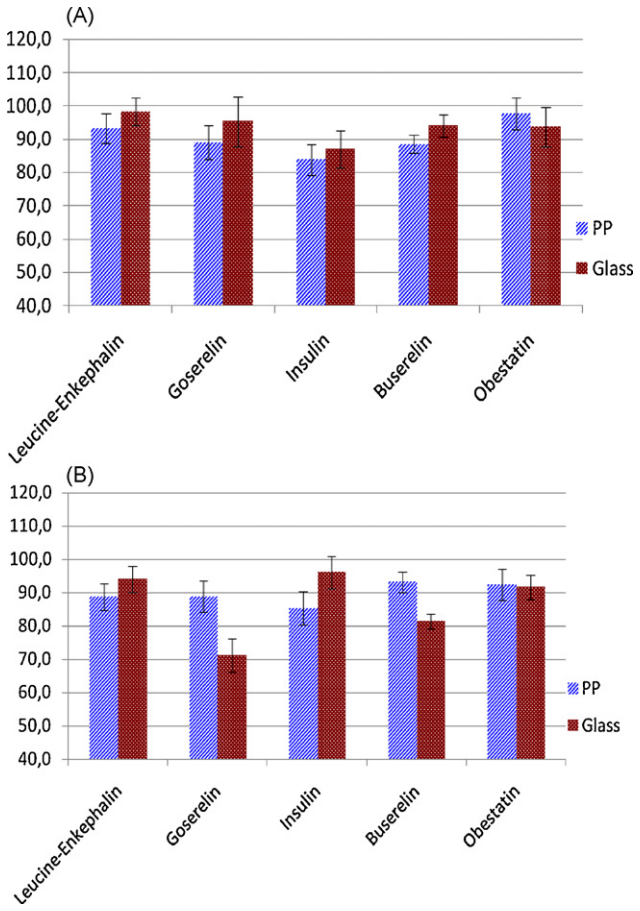


Fig. 4. Individual peptide (*i.e.* leucine-enkephalin, goserelin, bovine insulin, buserelin and mouse obestatin) effect on drying recovery (%) in two materials, *i.e.* glass and polypropylene (PP) using Speedvac (A) and Lyovac (B) (mean bar plots ± S.E.M.).

and additives), bovine insulin showed the smallest recovery value (85.0%; $P = 0.0001$) when compared to the other peptides: leucine-enkephalin (93.6%), goserelin (86.8%), buserelin (89.2%) and mouse obestatin (93.6%). It is observed that the increased adsorption for the model peptides onto glass versus PP when lyophilization was applied (see above) is more pronounced for buserelin and goserelin (*i.e.* the two GnRH agonists with similar sequence). Likewise, Grohganz et al. [8] demonstrated that a considerable amount of cetrorelix (*i.e.* a GnRH antagonist) was adsorbed onto the walls of glass vials. Although in general, the recovery of Lyovac-dried model peptides in PP was higher than in glass (see Fig. 2), this was not the case for leucine-enkephalin and bovine insulin (see Fig. 4B). In general, the variability in adsorption between the different peptides in glass vials was higher than in PP vials. Initial QSPR modelling, using algorithms as previously described [18], indicates that the molecular shape of the peptide is thought to be an important determinant: descriptors describing the peptide molecular shape such as PW2 (path/walk 2-Randic shape index) or WHIM (Weighted Holistic Invariant Molecular) first component shape directional P1 indices (unweighted as well as weighted by atomic van der Waals volumes) indeed show a high correlation with the glass adsorption.

4. Conclusions

The results of the present study demonstrate that a drying step (*e.g.* as part of the sample preparation) can cause significant peptide loss. Introducing suitable additives and the application of suitable container materials can avoid or diminish the peptide loss during solvent evaporation. The use of C_9-Glu improved the recovery of model peptides in both Speedvac and Lyovac methods. PEG 400 and mannitol were found to increase peptide adsorption. Moreover, PEG 400's presence resulted in even reduced recoveries for all model peptides when using Speedvac in combination with PP vials. Maximum amounts of model peptides were recovered for the following vial type - evaporation technique combinations: polypropylene – lyophilization and glass – centrifugal evaporation. Thus applying C_9-Glu as an additive along with choosing appropriate vial material can prevent or decrease peptide loss during the selected solvent removal evaporation procedure.

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