

Tips and tricks of the trade

Hidden Peptide Losses

Many researchers don't give enough consideration to the material of their tubes and containers they use for handling and storage of peptides. This may have dire consequences.

Lab Hint

This is a laboratory tragedy – a tale of loss and deception, a fatal attraction and a ruined career. No, I'm not talking about the latest episode of your favourite medical drama. I am talking, of course, about peptide losses caused by surface adsorption. Here is the problem: you are trying to measure concentrations of peptides accurately, however, the stuff is sticky and every time you move it from one container to another, some gets lost. So your measure-

running. This is the last chance to get the materials in on time if the experiment is going to get done next week. A morning spent getting lost in online catalogues – plastic eppendorfs, glass beakers, a homogenizer, oh and don't forget those separation kits with those neat little plastic cartridges. "And I think I'll need more cell culture tubes. Now, let me see – okay, according to the catalogue I can have them in no less than three different types of plastic or two different types of glass. What a pain. Which plastic should I choose? Well, I guess it doesn't matter – the polypropylene ones

paper is sent off; the referees are cautiously impressed; Paul is interviewed on the BBC. But doom draws near. If only Paul hadn't used the polypropylene tubes and the polystyrene tubes for the two different conditions. If only peptide-YY didn't stick nearly seven times more to polypropylene than to polystyrene. If only Paul had read a paper by Goebel-Stengel and her co-authors from the Martin Luther Hospital in Berlin, that came out earlier this year (*Analytical Biochemistry*, 1;414(1):38-46).

It is not comfortable reading for anyone who really needs to know protein concentrations accurately. The fact is, proteins love plastic: peptide-YY has a penchant for polystyrene, nefstatin favours flint and ghrelin is a slave to glass. And just like humans, a protein's bonding preference is highly unpredictable.

Shocking results

Goebel-Stengel *et al.* took eight different peptides of interest to the field of endocrinology and kept them in different tubes for 48 hours. They then measured the recovery of the radioactively-labelled peptides. The results were shocking. Overall, recovery of the peptides stored in different containers varied from 90% down to less than 10%. The storage material affected some peptides more than others: CRF was barely affected by the material (although 40% of the peptide got stuck somewhere), whereas the loss of insulin varied sevenfold.

Like mixing drinks, Paul's mixed plastics were fatal – Goebel-Stengel *et al.* found that 40% of peptide-YY was lost when they used polypropylene, whereas using polystyrene meant that about 90% of the peptide was lost. Even if Paul had stuck to the same plastic (sorry about the pun) he wouldn't have been much better off. What works for one protein won't work for another: Ghrelin sticks to either form by about the same amount.

As Goebel-Stengel and Co. points out, in a typical, routine laboratory assay a peptide sample will have passed from a glass homogenizer through eppendorf tubes and separation cartridges before finally being



Some peptides stick as stubbornly to the surface of glass or plastic labware as chewing gum to shoe soles.

ments are out. But it is worse than that – some peptides are stickier than others and they stick to different plastics to different degrees.

Tragic story

Shakespeare couldn't have penned a better tragedy. The plot outlined: It is early on a Wednesday morning; Paul the Postdoc buys in the materials for his assay he's

are the cheapest. Hang on; the polystyrene ones come in a smaller pack. I think I'll get a pack of each."

Come next week, the crucial assay is run, the results discussed. Paul revels in the approval of his colleagues as he shows them the data: condition A, 10 nanomoles of protein; condition B, 100 nanomoles. Yes, the lab's favourite protein is present in different amounts in the two conditions. The

assayed. And think of vigorous vortexing: you'd think it was just purpose-designed to bring the peptide into sticking distance of the plastic. If anything, between 10 and 90% is lost at each step – well, I'll leave the maths to you.

In fact, it is the unpredictability of the problem that is the most alarming part of Goebel-Stengel's findings. They chose their peptides carefully so that some were basic, some acidic and some uncharged – none of these factors produced any clear correlation with how much the peptides were adsorbed. In fact, knowing about the molecular properties of the peptides and the physics of the surfaces gets you nowhere. You would have thought that ghrelin, with its hydrophobic side chain, would have shown a taste for the organically-based plastic surfaces, but no – it actually preferred glass.

Siliconizing doesn't help

"Oh but I'm okay", I hear you say. "I always siliconize my glass". Sorry, but that news isn't good. Siliconizing actually increased the loss of all but two of Goebel-Stengel's peptides. For the remaining two it had no effect, at all.

Goebel-Stengel is not alone in raising the hue and cry. Two years ago, Alexandra Kraut reported different effects of plastics and glasses on the storage of peptides, concluding that standard plastics are just not at all suitable for peptide storage (Kraut *et al.*, *Journal of Proteome Research* 2009 8 (7), 3778-85). Lindberg and Andersson as far back as 1991 warned that up to 73% of atrial natriuretic peptide was lost to plastic adsorption (*Pharmacol Toxicol.* 1991 Apr;68(4):276-81). Meanwhile, there is increasing awareness that the use of microfluidics using narrow-bore plastic conduits seems almost designed to create adsorption problems (Laven *et al.*, *Lab Chip*, 2005, 5, 756-63).

Old problem

Peptides sticking to the vessels we keep them in is nothing new and seasoned peptide researchers are only too aware of the dangers. The website of the UK's National Institute for Biological Standards and Control (NIBSC), a centre for the Health Protection Agency, dedicates a whole paragraph to the choice of a container for storage of peptides, warning that "peptides in solution can and do adsorb to many materials" and that "it is possible to lose large percentages of peptide due to adsorption to surfaces, thereby grossly distorting subsequent results" (www.nibsc.ac.uk/spotlight/cjd_re

[source_centre/available_samples/peptide_library/peptide_storage.aspx](http://www.nibsc.ac.uk/spotlight/cjd_re/source_centre/available_samples/peptide_library/peptide_storage.aspx)).

And Paul might have been lulled into complacency by a slurry of reports suggesting that it really doesn't matter what material you use. Clinicians have looked quite carefully at whether container composition affects samples. The consensus is: plastic or glass, it's up to you. But they are dealing with comparatively highly concentrated forms of protein, for instance blood samples. The problem comes when your peptide is heavily diluted.

What can you do?

So, given the alarming news, how can you avoid repeating Paul's tragic tale of loss and illicit bonding?

Well, if Goebel-Stengel's lab is right, strictly speaking you have to run a recovery assay for every material you use. This will be inevitable in experiment designs that compare the concentrations of different peptides.

But there are things that can be done to reduce peptide losses and avoid artefacts.

► In experiment designs, which compare concentrations of different peptides, repeat the comparison using two different sets of plastics/glasses. If the materials are chosen to be as divergent as possible, artefacts caused by different rates of peptide loss are more likely to show up in an increased variability in the results, i.e. incorporate a cross comparison element into the experiment design.

► In experiment designs that compare treatments on the same peptide, be consistent in which products you use (even to using tubes from the same manufacturer's lot).

► Add BSA (bovine serum albumin) to your mix. Goebel-Stengel *et al.* found that BSA consistently improved peptide recovery – to nearly 100% for most peptides for the best material. In the cheery words of Goebel-Stengel, "This is as much as can be recommended using the current data."

Oh, and if you *do* happen to see Paul, spare him a few coins as it's getting cold out on the streets, right now. And whatever you do, don't mention protein recovery to him. It's a bit of a sticky subject.

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Do you have any useful tips?

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Lab Times

Founded 2006. Issue 7, 2011
Lab Times is published bimonthly

ISSN: 1864-2381

Publisher: LJ-Verlag Herfort und Sailer
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Printed at:

Stürtz GmbH,
Alfred-Nobel-Straße 33, 97080 Würzburg, Germany

Web:

www.lab-times.org
Webmaster: Carsten Rees
Tel.: +49(0)761-1563461, webmaster@lab-times.org

Prices & Subscription rates:

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