**Manuscript No.: JoVE55086R2**

**Title: Evaluation method for drug sorption to PVC- and non-PVC-based tubes in administration sets using a pump**

**Response for comments:**  
  
Thank you for the careful Peer Review revisions that were made to the manuscript. The following editorial comments need to be addressed.  
  
• Your manuscript has been modified by your editor, please maintain the current formatting throughout the manuscript. Please use the updated manuscript located in your Editorial Manager account (under “File Inventory”) for all subsequent revisions. The updated manuscript is also attached.

***• 1.3: How are the tubes cut precisely? Sharp razor?***  
→ Recommendation added.

**PROTOCOL**

1. Preparation of tubes in administration sets

Note: Perform the cutting step precisely to eliminate the effect of differences in tube length on drug sorption. Use a sharp razor.

***• 2.1: Please re-write for grammar and clarity.***

2.1) Label the bottle with the dame (e.g., diazepam and tacrolimus).

→2.1) Add a label indicating drug name (e.g., diazepam or tacrolimus) to the bottle.

***• 2.2. Note: Please re-write for grammar and clarity.***  
2.2) Dilute drug injections to the following concentrations in bottled 5% dextrose solution: diazepam injections, 1 mg/mL to 100 μg/mL (10 mL of diazepam injection in 100 mL) and tacrolimus injections, 5 mg/mL to 10 μg/mL (200 µL of tacrolimus injection in 100 mL)

→2.2) Dilute drug injections with 5% dextrose solution: from 1 mg/mL to 100 μg/mL for diazepam injections (10 mL of diazepam injection in 100 mL of 5% dextrose solution) and from 5 mg/mL to 10 μg/mL for tacrolimus injections (200 µL of tacrolimus injection in 100 mL of 5% dextrose solution).

***• Protocol section 3.2: Please clarify how to insert the middle of the preloaded tube into the infusion pump. It is not clear what the “middle” of the tube indicates.***

→To clarify the protocol 3.2, we added the inside of an infusion pump in Figure 2 and corrected the unclear part.

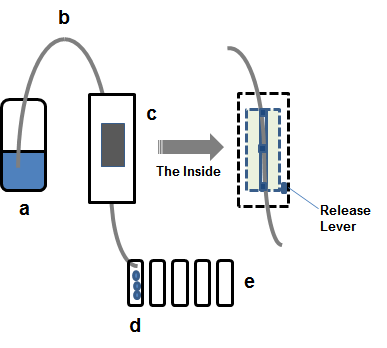


Figure 2. Test set of kinetic sorption study using a pump. (a) Drug diluted with 5% dextrose in a bottle, (b) tube of administration set (1 m in length), (c) infusion pump, (d) drug passed through the tube, and (e) amber vials for storage. To minimize additional drug sorption, drug solutions and samples were prepared and stored in a glass bottle and amber vials for injections, respectively. This figure has been modified1.

***• 3.2.4: What does the glass graduated cylinder contain?***  
→The glass graduated cylinder is made of chemically resistant borosilicate glass and used to collect the drug solutions without additional sorption to polymeric materials.

3.2.4) Put the end of tubes into a glass graduated cylinder after removing the syringe.

→3.2.4) Put the end of tube into a chemically resistant borosilicate glass graduated cylinder, which is used to collect drug solution after passing through the tube.

***• Figure 4 legend: Please clarify “In the chromatogram, solvent peak was presented before diazepam peak”.***

**Figure 4. Representative chromatograms of diazepam.** (a) Blank (methanol), standard at (b) 0.3125 µg/mL and (c) 20 µg/mL, and (d) sample in 5% dextrose. The peak of diazepam was detected at 8.2 min and fluctuations in retention time occurred within 1 min in the chromatograms. In the chromatogram, solvent peak was presented before diazepam peak.

→**Figure 4. Representative chromatograms of diazepam.** (a) Blank (methanol), standards at (b) 0.3125 µg/mL and (c) 20 µg/mL, and (d) sample (5% dextrose). The peak of diazepam was detected at 8.2 min, and fluctuations in retention time occurred within 1 min in the chromatograms. The peaks from solvent were presented before the diazepam peak in the chromatograms of blank and standard.

***• Table 2: Please provide at least replicate data. Data from previous publication is acceptable.***  
→We added the data in Table 2. Results are expressed the mean ± SD (n=3).

**Table 2. Representative sorption results for diazepam and tacrolimus in PVC- and non-PVC-based tubes (n=3)1.**

|  |  |  |  |
| --- | --- | --- | --- |
| Drug | Sorption (%) | | |
|  | PVC | PU | PO |
| Diazepam (0.05 h) | 27.6 ± 3.0 | 21.9 ± 12.6 | 11.3 ± 4.6 |
| Tacrolimus (1.05 h) | 15.1 ± 3.3 | 10.3 ± 6.3 | 0.6 ± 0.9 |

***• Please expand your discussion to cover the following in detail and in paragraph form: 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol. Several points have been deleted and must be added.***   
→We clarify the limitations of the technique, significance with respect to existing methods, future applications and critical steps within the protocol.

**DISCUSSION**

Drug sorption to administration sets is a cause of unexpected drug loss in intravenous drug delivery. During sorption, drugs are generally partitioned to polymeric materials of tubes at the early phase of infusion; after sorption equilibrium is reached, the delivered amount of drugs is stabilized1. The sorption levels of drugs should be evaluated and minimized. Several evaluation methods for drug sorption have been studied, such as a pump method and a drip method. Compared to the drip method, the pump method can be easily manipulated without bias. Although administration sets with flow regulators (conventional form) are used in the drip method, desired flow rates less than 5 mL/h are hard to achieve. Therefore, we recommend the pump method for sorption evaluation of tubes in administration sets***.(3) significance with respect to existing methods)***

When using the pump method, major factors that affect drug sorption to tubes of administration sets are classified by drug properties (e.g., hydrophobicity, charge), conditions of sorption test (e.g., drug concentration, flow rate, solvent compatibility, tube length, temperature), analytical methods for drugs (e.g., HPLC, MS), and polymers of tubes in the administration sets (e.g., PVC, PU, PO)2-12.***(5) critical steps within the protocol)*** First, selection of the model drugs is critical for obtaining precise and accurate experimental results. Even though diazepam management is tracked by the Psychotropic Drugs Control Act, we selected diazepam (Figure 1a) and tacrolimus (Figure 1b) as model drugs because of their high sorption levels to polymeric tubes of administration sets1 or containers13, 14. In this case, drugs at high concentrations showed less sorption than those at low concentrations in the early phase of infusion1, 2. These drugs have high log P values (diazepam - 2.8215, tacrolimus - 3.9616) and low solubility as categorized by the Biopharmaceutical Classification System (BCS class 2). Because of their hydrophobicity, these drugs can interact with tubes of administration sets, leading to sorption. Other drugs showing high sorption levels (e.g., nitroglycerin2 and cyclosporin A3) can be used as alternative model drugs for sorption evaluation. Furthermore, macromolecular drugs such as biologics (antibody therapeutics, insulin, etc.) can be applied for quality evaluation of administration sets regarding drug sorption12. ***(1) modifications and troubleshooting)***

Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers.***(5) critical steps within the protocol)*** In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. **(*2) limitations of the technique)*** If a clinical condition requires it, we can use a multiplication factor for a length of tube.***(1) modifications and troubleshooting)*** In the sorption test, the diluted drug solutions were used as starting concentrations1,7. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model7. Comparing with double-lumen extension tube model17, diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs. ***(1) modifications and troubleshooting)***

In this protocol, we chose the HPLC method for drug analysis based on previous reports1, 8, 9. Simple and reproducible HPLC methods have been developed. The HPLC conditions are listed in Table 1. Various other techniques such as MS and immunoassay have also been developed as alternative analysis methods of drug concentrations10, 11. MS/MS and immunoassay are highly sensitive for detection of drugs and their metabolites. Specifically, immunoassay can be easily performed without requiring large and expensive equipment for drug analysis.***(1) modifications and troubleshooting)***

Regarding quality evaluation of administration sets, drug sorption to PVC- and non-PVC-based materials used in the tubes of administration sets has been studied. Evaluation of sorption to tubes in administration sets started with drug selection and ended with consideration of acceptable criteria of sorption levels as illustrated (Figure 3). PVC-based tubes showed high sorption levels for many drugs such as diazepam, tacrolimus (Table 2), nitroglycerin2, and cyclosporin A3. Among approaches to minimize drug sorption in the tubes of administration sets to less than 10%, alternative materials or designs of polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs2, 13. PE/PB/PP blended PO-based tube of administration set used in this study, showed low sorption level as non-PVC-based tube. On the other hand, PE-based tubes are not used for the administration sets, but commercially used in the market as a syringe extension tube due to their hardness.

This protocol can be applied for the quality control of administration sets with respect to drug sorption. More drugs classified by sorption level (highest, lower, and lowest) should be used in sorption evaluation for quality assurance of administration sets. This protocol can also be used in scientific research for development of new alternative polymeric materials or new designs for tubes in administration sets that do not result in drug sorption1, 13.***(4) future applications)***

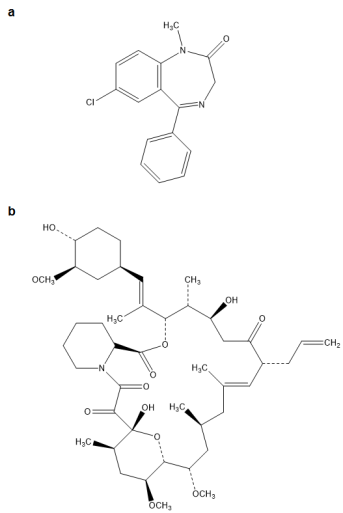
***• Please copy edit your manuscript. There are a number of grammatical errors throughout. We recommend that you have a native English speaker copy-edit the manuscript.***  
→We have an English editing service with a native English speaker. Grammatical errors have been corrected.

• ***Please discuss the limitations of the method in the Discussion section.***  
→We discussed 2) limitations of the method as mentioned above.

**DISCUSSION**

~. In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, we can use a multiplication factor for a length of tube.~

• ***Figure 1: Is this Figure from Wikipedia? If you are re-using figures (e.g. Figures 1 and 2) from a previous publication, please obtain explicit permission to re-use the figure from the previous publisher (this can be in the form of a letter from an editor or a link to the editorial policies that allows you to re-publish the figure). Please upload the text of the re-print permission (may be copied and pasted from an email/website) as a Word document to the Editorial Manager site in the "Supplemental files (as requested by JoVE)" section.***→We redraw chemical structures of diazepam and tacrolimus.



**Figure 1. Chemical structures of model drugs: (a) diazepam and (b) tacrolimus.** Diazepam is a benzodiazepine derivative, and tacrolimus is a 23-membered macrolide lactone. This figure has been modified1.

***• Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please address and/or expand on the following comments in the manuscript:***

**Reviewer #1:**  
***• The main part step 3.2 is not clear enough. Subsequently the related figure (#2) is not well illustrated. The "middle part" is nonsense and also after "removing the syringe" the open end is left inside the drug solution or what?***  
→To clarify the protocol 3.2, we added the inside of an infusion pump in Figure 2 and corrected the unclear part as mentioned above.

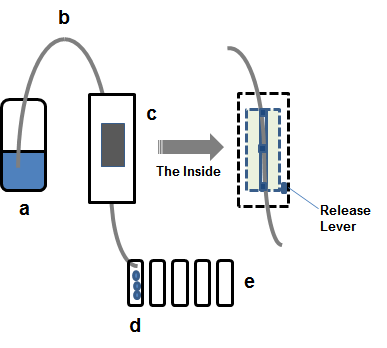


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***• Step 4.3.1: Not all drugs can be quantified using UV detector. It should be considered here.***  
→We added the note for the detection method.

4.3) Analyze standards using the HPLC method with UV detection1, 8, 9.

Note: Use an appropriate detection method (UV, fluorescence, etc) for drug analysis.

**Reviewer #2:**  
***• The sorption model proposed by Su-Eon Jin et al isn't an innovative concept. Provided data are inadequate in view of recent literature on the subject, e.g. the article of Foinard et al (PlosOne, 2016). This model doesn't correspond to a standardized method or guidelines because it couldn't be applied to any drug solution. It has been specifically performed on 2 drugs wellknown to absorb in PVC material : diazepam and tacrolimus, in very specific clinical conditions. The latter may be very different according the drugs tested. Here, specific parameters are tested:  
- administration procedure : with a pump procedure,using a syringue pump  
- length of tubes (which could influence the sorption)  
- flow rate : because they are plenty of different flow rates used in clinical practice (especially considerating adults or children patients and also considerating the indication)  
- specific tested concentrations: they may represent the therapeutic use of drugs. If several dosings do exist according the indication, they all have to be tested (especially for diazepam in this study)  
- the acceptable criteria of drug sorption (±10%) : nowadays, some drugs' stabilities have to match an acceptable range less than 5% beacause of the physicochemical nature and the toxicty of the drugs. Such is the acs for instance of anticancer agents, as it is recommended in the last SFPO (Société Française de Pharmacie Oncologique) guidelines (Bardin et al, Annales Pharmaceutiques Françaises 2011)  
- etc…  
\*This comment was not satisfactorily addressed in the manuscript. Please discuss the points made in the rebuttal letter in the manuscript. Please note that the authors’ rebuttal was not clear and did not address the reviewer’s comments.***→ Sorption model used in Jin et al. is already proposed by Roberts (reference: 7. Roberts, M.S. Modeling solute sorption into plastic tubing during organ perfusion and intravenous infusions. *J. Pharm. Sci.* **85**, 655-665, doi:10.1021/js9500621 (1996).). This model is designed as nonlinear regression with a convection-interfacial resistance-diffusion based on the preloaded tube model with solution. This model has been confimed. We also checked the test conditions (Jin et al.). We used this model to explain our data set and confrimed whether the pattern was matched or not.

The model proposed by Foinard et al. is not new. Foinard et al. didn’t consider the initial point of drug concentration before delivery in sorption study. If they consider the concentration of drug at real "0" time of starting point, their concentration level should be started with 100%. Then, it is the same as Roberts. We think the model from Roberts can correspond to a standardized method or regulatory guidelines because sorption level should be compared with the drug concentration at starting point.

Although diazepam and tacrolimus are highly sorptive drugs categorized by BCS class II, their sorption levels are different in various types of tubes. Specifically sorption levels of diazepam and tacrolimus are lowered in PO-based tubes of administration sets than other tubes of administration sets. We recommended the protocol to minimize the affecting factors for drug sorption by fixing several parameters. This protocol is designed to perform easily. The acceptable criteria of drug sorption using our protocol with recommended drugs were lower than 10%. In the case of specific drugs, it should be included in pharmacopoeia or prescribing information.

→ We added the reviewer’s comment in protocol and discussion of the manuscript.

**REPRESENTATIVE RESULTS**

~The recommended acceptable range of drug sorption percentages was less than 10%, based on the content of injections from pharmacopoeias12. In addition, the specific drugs (e.g., anticancer drugs) should be confirmed with clinical guidelines.~

**DISCUSSION**

~ When using the pump method, major factors that affect drug sorption to tubes of administration sets are classified by drug properties (e.g., hydrophobicity, charge), conditions of sorption test (e.g., drug concentration, flow rate, solvent compatibility, tube length, temperature), analytical methods for drugs (e.g., HPLC, MS), and polymers of tubes in the administration sets (e.g., PVC, PU, PO)2-12. First, selection of the model drugs is critical for obtaining precise and accurate experimental results. Even though diazepam management is tracked by the Psychotropic Drugs Control Act, we selected diazepam (Figure 1a) and tacrolimus (Figure 1b) as model drugs because of their high sorption levels to polymeric tubes of administration sets1 or containers13, 14. In this case, drugs at high concentrations showed less sorption than those at low concentrations in the early phase of infusion1, 2. These drugs have high log P values (diazepam - 2.8215, tacrolimus - 3.9616) and low solubility as categorized by the Biopharmaceutical Classification System (BCS class 2). Because of their hydrophobicity, these drugs can interact with tubes of administration sets, leading to sorption. Other drugs showing high sorption levels (e.g., nitroglycerin2 and cyclosporin A3) can be used as alternative model drugs for sorption evaluation. Furthermore, macromolecular drugs such as biologics (antibody therapeutics, insulin, etc.) can be applied for quality evaluation of administration sets regarding drug sorption12.

Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers. In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, we can use a multiplication factor for a length of tube. In the sorption test, the diluted drug solutions were used as starting concentrations1,7. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model7. Comparing with double-lumen extension tube model17, diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs.

In this protocol, we chose the HPLC method for drug analysis based on previous reports1, 8, 9. Simple and reproducible HPLC methods have been developed. The HPLC conditions are listed in Table 1. Various other techniques such as MS and immunoassay have also been developed as alternative analysis methods of drug concentrations10, 11. MS/MS and immunoassay are highly sensitive for detection of drugs and their metabolites. Specifically, immunoassay can be easily performed without requiring large and expensive equipment for drug analysis.

Regarding quality evaluation of administration sets, drug sorption to PVC- and non-PVC-based materials used in the tubes of administration sets has been studied. Evaluation of sorption to tubes in administration sets started with drug selection and ended with consideration of acceptable criteria of sorption levels as illustrated (Figure 3). PVC-based tubes showed high sorption levels for many drugs such as diazepam, tacrolimus (Table 2), nitroglycerin2, and cyclosporin A3. Among approaches to minimize drug sorption in the tubes of administration sets to less than 10%, alternative materials or designs of polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs2, 13. PE/PB/PP blended PO-based tube of administration set used in this study, showed low sorption level as non-PVC-based tube. On the other hand, PE-based tubes are not used for the administration sets, but commercially used in the market as a syringe extension tube due to their hardness. ~

***• Administration sets in polyethylene have not be tested here. Why? If such material is marketed, it should also be evaluated for drug sorption. Please mention the details presented in the rebuttal letter in the Discussion section. It is not clear what the authors are trying to convey here.***→ Polyethylene (PE)-based tubes are not currently available in the market of administration sets because only PE-based tubes are too hard to use as tubes of administration sets. They are used in syringe extension sets. We used blended PO tubes of administration sets.

**DISCUSSION**

~ Among approaches to minimize drug sorption in the tubes of administration sets to less than 10%, alternative materials or designs of polymeric combinations have been developed, such as PO-based materials and layer-by-layer designs2, 13. PE/PB/PP blended PO-based tube of administration set used in this study, showed low sorption level as non-PVC-based tube. On the other hand, PE-based tubes are not used for the administration sets, but commercially used in the market as a syringe extension tube due to their hardness.~

***• The administration sets are cut to 1m length. Why? It doesn't reflect clinical conditions because the devices often are more than 1.5m length: Author response (from the rebuttal letter): To remove the accessories of administration sets except tubes, we cut the tubes. If we use shorter length of tubes like 1 m in length, it takes shorter time to evaluate the drug sorption to tubes of administration sets. For considering a length factor of tube, it can reflect clinical conditions. Overall, it makes a protocol simple and easy to perform based on the clinical conditions. This comment was not satisfactorily addressed. Is the tube cut to 1 m for simplicity and easy of performance? In that case, it does not reflect the clinical settings.***  
→We already mentioned the length of tubes for sorption test. The tube cut to 1 m is not a clinical setting, but it can be easily multiplied for the total length of tubes to reflect the clinical settings. We mentioned it in discussion.

**DISCUSSION**

~Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). ~ In this study, tubes without other accessories at a fixed length of 1 m were used to simplify the factors of drug sorption to tubes of administration sets. If a clinical condition requires it, we can use a multiplication factor for a length of tube. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e).~

**• *There is no kinetic study (results) as mentioned by the authors. The results show only a percentage value of sorption at the end of the study. Values at each kinetic time have to be presented (table or graph).* Please elaborate on your published kinetic study in the results section. Please provide the reference.**  
→We already provided the kinetic data for sorption study (Jin et al., 2016). Highest sorption level from the previous study (Jin et al., 2016) showed in the early phase of kinetic study. Thus, we provided the initial sorption levels of the drugs as representative results. We added the reference in Table 2 and mentioned the kinetics in discussion.

**REPRESENTATIVE RESULTS**

**Table 2. Representative sorption results for diazepam and tacrolimus in PVC- and non-PVC-based tubes (n=3)**1**.**

|  |  |  |  |
| --- | --- | --- | --- |
| Drug | Sorption (%) | | |
|  | PVC | PU | PO |
| Diazepam (0.05 h) | 27.6 ± 3.0 | 21.9 ± 12.6 | 11.3 ± 4.6 |
| Tacrolimus (1.05 h) | 15.1 ± 3.3 | 10.3 ± 6.3 | 0.6 ± 0.9 |

**DISCUSSION**

Drug sorption to administration sets is a cause of unexpected drug loss in intravenous drug delivery. During sorption, drugs are generally partitioned to polymeric materials of tubes at the early phase of infusion; after sorption equilibrium is reached, the delivered amount of drugs is stabilized1. ~ Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model7. Comparing with double-lumen extension tube model17, diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs.~

**Reviewer #4:**  
**• *At any point during the process, are you testing the starting concentration of the drug solution in the glass bottle? If not, this should be included as a baseline (control).* Author response: We tested the starting concentration of drug solution in Jin et al. 2016. We collected the samples after drug dilution in a bottle and analyzed. Please mention if and when the starting concentration of drug solution is measured in the JoVE manuscript results section. Please provide the reference**.

→Thank you for the comments. We used the starting concentration of the drug solution in a glass bottle as a control. We also clarified the results from protocol 5.2. References are added in the discussion.

**REPRESENTATIVE RESULTS**

~ All samples including samples at starting points were analyzed using an HPLC method with UV detection (Figure 3a). Analysis conditions are listed in Table 1. ~ The drug concentrations at starting points of the sorption study were calculated from the analysis of samples after drug dilution. Sorption levels in PVC- and non-PVC-based tubes of administration sets were determined by calculating the percentage of remaining drug content after passage through the tubes from the calibration curves (Figure 3b) and subtracting these values from 100% (Figure 3c).~

**DISCUSSION**

~ In the sorption test, the diluted drug solutions were used as starting concentrations1,7. After delivery, the drug solution (Figure 2d) was collected into vials at various time points (Figure 2e). Drug solutions for sampling were passed completely through the entire tube at the preselected conditions of flow rate and sampling time points. Sorption generally occurs in the early phase of infusion and the pattern is followed by convection-interfacial resistance-diffusion model7. Comparing with double-lumen extension tube model17, diazepam sorption results are comparable when the initial drug concentration is considered before delivery. Therefore, sampling time points can be modified so that sorption evaluation takes less time. All factors of test conditions were confirmed based on the clinical usage of drugs.~

***• Was the glassware used in this experiment silanized or otherwise coated? If not, are there any scenarios in which it may need to be? Please address this comment in the Discussion section.***

→The glassware was made of chemically resistant borosilicate glass. We mentioned it in the manuscript at protocol 3, specifically 3.2.4 and discussion section.

**PROTOCOL**

~3. Kinetic sorption study using an infusion pump

Note: Confirm tube-dependent flow rate using a pump prior to the sorption test due to the hardness of tubes. Collect samples at precise time points and use glass bottles and vials to prevent additional drug sorption during storage. Perform the test as shown in Figure 2. Protect the drug solution against light if the drug has photosensitivity. Perform the experiments in triplicate.

3.1) Without creating air bubbles, preload a diluted solution of the drug into the tube using a syringe.

3.1.1) Connect one end of the tube to a syringe.

3.1.2) Put the other end of the tube into the bottled drug solution.

3.1.3) Pull back the syringe plunger until the tube is completely filled with the drug solution.

3.2) Install the tube into an infusion pump.

3.2.1) Open the door of the infusion pump and push the release lever.

3.2.2) Insert the middle of the preloaded tube into the infusion pump and keep it straight.

3.2.3) Remove the syringe at the end of the tube after installation of tube.

3.2.4) Put the end of tube into a chemically resistant borosilicate glass graduated cylinder, which is used to collect drug solution after passing through the tube.~

**DISCUSSION**

~Next, we set up a simple kinetic sorption study using a pump to easily obtain the precise results, and minimize artifacts. (Figure 2). In the pump method, drug solution (Figure 2a) was passed through a tube cut from the administration set (Figure 2b) after installation into an infusion pump (Figure 2c). Except for the tubes from administration sets, all devices (bottle, graduated cylinder, and sampling vials) were composed of chemically resistant borosilicate glass to prevent additional drug sorption to polymers.~

***• Step 4.2.1: The authors use diazepam stock solutions and standards for calibration that are made up in methanol, but are testing sample concentrations of diazepam made up in 5% dextrose. Please comment on the different solvents and what differences might be expected in HPLC chromatograms and/or quantitation of diazepam concentration. Author response: We already checked the diazepam concentration in samples using calibration curves from diazepam standards. Diazepam standards were insoluble in 5% dextrose, so they were dissolved in methanol and diluted with methanol. In samples, using a different solvent from standards, other interfering peaks may be presented in the chromatograms. In this case, although there are peaks at 4 - 6 min before diazepam peak, they were not overlapped to diazepam peak in the chromatogram. Please discuss your response in the Results or Discussion section.***

→We added the response in results.

**REPRESENTATIVE RESULTS**

**~** Analysis conditions are listed in Table 1. For the preparation of drug standards, diazepam and tacrolimus were dissolved in methanol and acetonitrile because of their insolubility in 5% dextrose. **~** There were no interfering peaks from the matrix. Specifically in samples of diazepam sorption study, the interfering peaks were not overlapped to drug peak in the chromatogram although the different solvent (5% dextrose) was used from standards.~