

1. Introduction

Automated patch-clamp systems have dramatically improved efficiency of experiments to detect drugs' potency of QT prolongation. However, it has become clear that the experimental results of some positive-control substances in the automated patch-clamp system, PatchXpress 7000A, differ from those in manual patch-clamping. Dose-response curves of the compounds obtained in the automated patch-clamp system shifted in the higher-concentration region than those in manual patch-clamping. This fact may affect the reliability of studies using the automated patch-clamp system, because the suppressive potency of a compound could be underestimated.

In this research, we investigated the possibility of sabstances' adsorption into 96-well plates used to store test-substance solutions in the automated patch-clamp system. As results, the concentrations of certain test substances in the 96-well plate made of polystyrene remarkably decreased in a time-dependent manner. Subsequently, we examined other 96-well plates made of various materials and found that the timedependent decrease of concentration in a glass-vial plate is much smaller than those in 96-well plates made of synthetic resins.

2. Methods

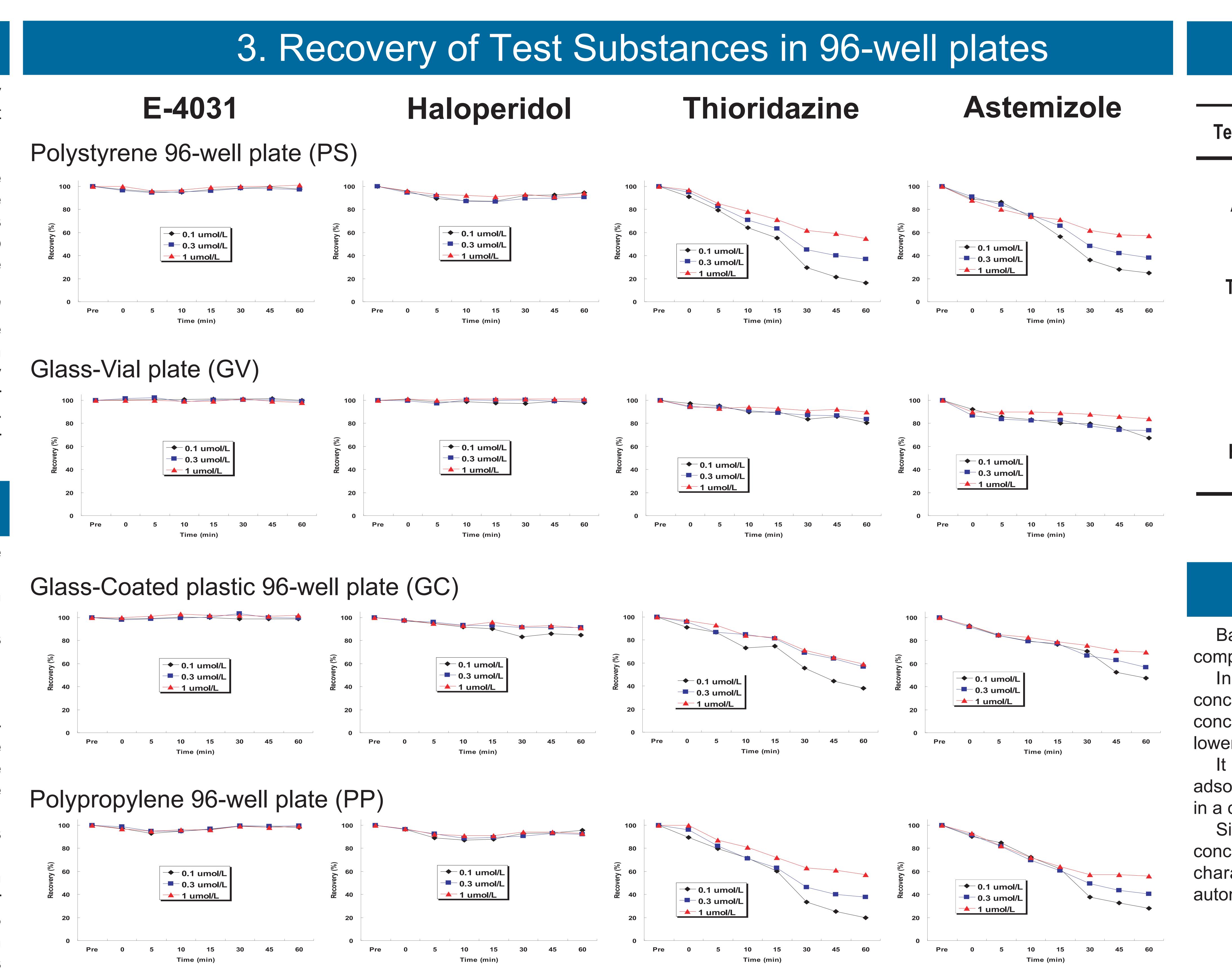
In this research, of the substances on which data differed from those from manual patch-clamping, astemizole and thioridazine were selected for examination of adsorption into 96-well plates; of the compounds on which data were equal to those from manual patch-clamping, E-4031 and haloperidol were selected. The 96-well plates are available in various materials; thus we selected glass-vial (GV), glass-coated plastic (GC), polypropylene (PP), and polystyrene (PS).

Astemizole, thioridazine, E-4031, and haloperidol were each dissolved in dimethyl sulfoxide (DMSO) to yield 1×10^{-4} , 3×10^{-4} , and 1×10^{-3} mol/L solutions. These solutions were diluted 1000 times with the superfusing solution and used as test solutions. A total of 300 μ L of the test solutions at the respective concentrations were placed in wells of the 96-well plate; 200 μ L of the test solutions were sampled. These sampled test solutions were mixed with 200 μ L of methanol, and used as samples for HPLC assay; the peak areas were determined.

A total of 8 sampling time points were set as follows: before placing in wells, right after placing (0 minute), 5, 15, 30, 45, and 60 minutes after placing. Sampling and determination of the peak areas were performed 3 times for each concentration. The peak area for samples before placing in wells was assumed as 100%; then a recovery rate for each sample was calculated based on their peak area for evaluation of adsorption level.

Compoud-Adsorption Problem and the Prevention Measure in the Automated Patch-Clamp Assay Naoto HONGO, Takayuki FUJITA, Kuniaki MATSUSHITA and Yuji TSURUBUCHI

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4.	Su	m	ma	N

est Substance	Conc. (uM) –	Recovery (%) *				
		PS	PP	GC	GV	
Astemizole	0.1	24.9	27.9	47.3	97.1	
	0.3	38.1	40.3	56.6	74.0	
	1	57.0	56.0	70.0	84.0	
Thioridazine	0.1	16.5	20.0	38.2	80.5	
	0.3	36.8	37.7	56.9	83.7	
	1	55.0	57.0	59.0	90.0	
E-4031	0.1	97.7	98.1	98.8	99.8	
	0.3	97.1	99.4	99.6	99.2	
	1	101.0	99.0	102.0	98.0	
Haloperidol	0.1	94.5	95.8	84.6	98.1	
	0.3	90.5	92.2	91.2	99.5	
	1	91.0	93.0	91.0	101.0	

* Recovery rate 60 minutes after placing in plates

5. Conclusion

Based on results described in the above, it was indicated that for some compounds the concentration extremely decreased due to adsorption.

In synthetic resins plates, recovery rates (indicating residual concentrations) were lower at the low concentration than those at the high concentration. It was suggested, therefore, recovery rates could be even lower, if the prepared concentration was lower than concentrations examined. It was also demonstrated that use of glass-vial plates could prevent this

adsorption problem to a certain degree and contribute to keep recovery rates in a certain level for a certain period.

Since the test results in this research differ depending on compounds or concentrations, it is recommended to select a material of the plate depend on characteristics of compounds when evaluating the compounds in the automated patch-clamp system like PatchXpress 7000A.