

Buffer plate preparation using ÄKTA™ avant system and BufferPro tool

Susanne Westin, Gustav Rodrigo, and Enrique Carredano
Cytiva, Björkgatan 30, 751 84 Uppsala, Sweden

Abstract

Manual buffer preparation for high-throughput process development (HTPD) requires a lot of effort as the number of different buffer conditions is large. A method was developed for automatic buffer preparation of 48-well buffer plates using ÄKTA™ avant chromatography system and the BufferPro tool in UNICORN™ 6 software. This is an alternative to preparing buffer plates with a robotic system. The 48-well buffer plate can be used together with PreDicator™ RoboColumn™ units, PreDicator™ plates, and Assist software.

Buffers can be made in the same way during the whole process development, from small-scale screening using PreDicator™ plates or PreDicator™ RoboColumn™ units, to laboratory-scale verification and optimization. Using BufferPro with ÄKTA™ avant gives the advantage of in-line pH and conductivity recording during the buffer preparation.



Screening/BufferPro

Verification/BufferPro

Scale-up and production

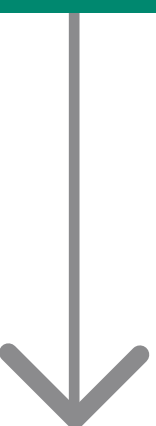
UNICORN™ method

The formulation of one buffer condition (pH/NaCl concentration) into one well

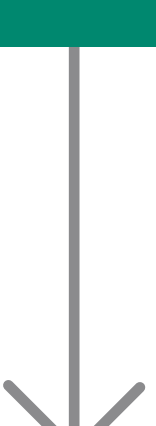
- Define buffer system in BufferPro, pH and NaCl concentration
- Prime flow system
- Fractionate the buffer into one well in the deep-well plate

UNICORN™ method

Define buffer system, pH and NaCl concentration



Prime flow system



Fractionate into one well

Result and conclusions

- All buffer systems included in BufferPro can be used in combination with 0 to 1 M NaCl (Table 1).
- pH and NaCl concentration per well can be varied. The chromatogram documents that the correct pH and conductivity is obtained in each well (Fig 1).
- Buffer plates can be prepared according to experimental design made with Assist software, to be used together with PreDicator™ plates.
- Time for preparing a 48 deep-well plate with 48 different buffer conditions is about 2 h at flow rate 25 mL/min., filling 4 mL per well.

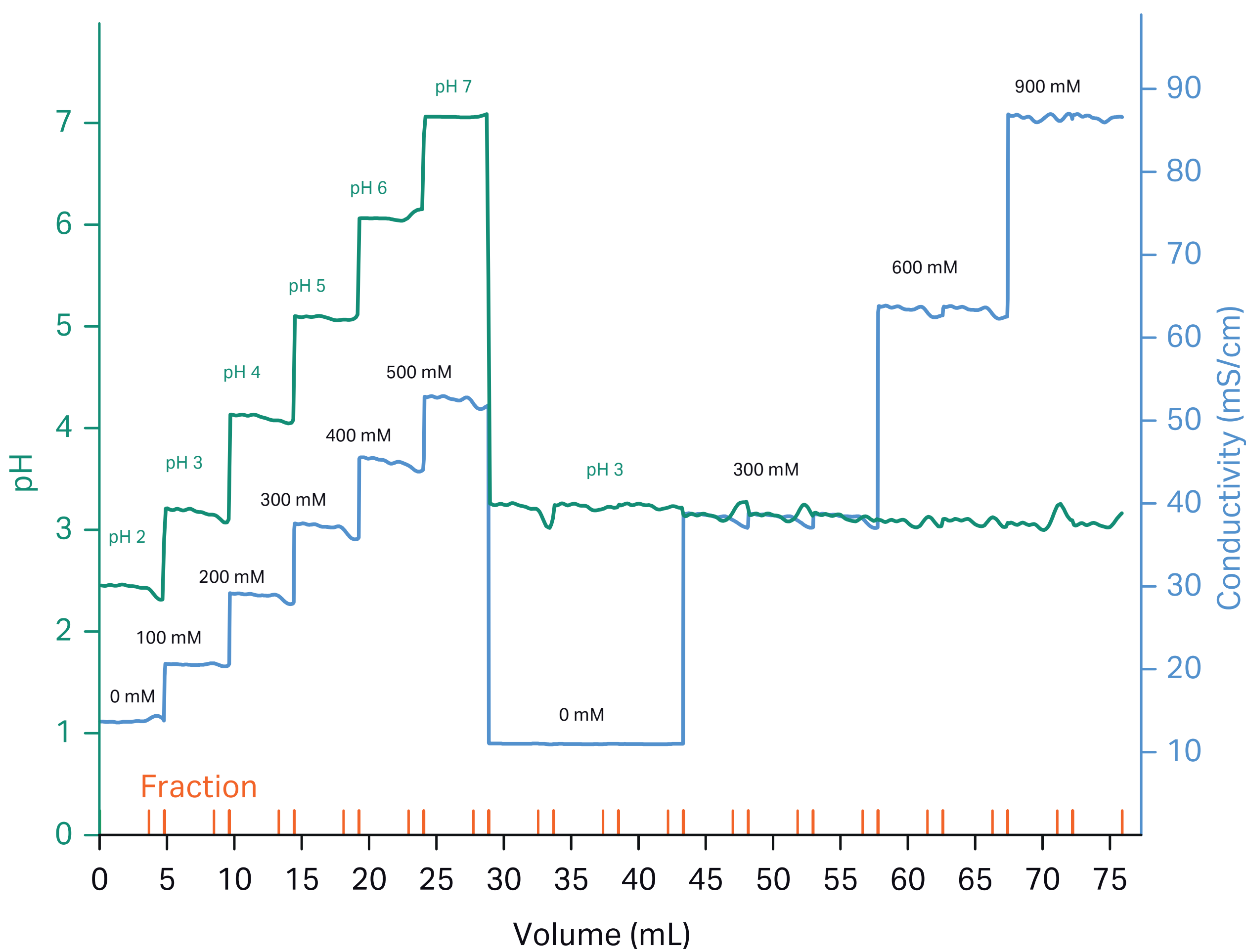


Table 1. Predefined buffer recipes included in BufferPro, from which new recipes can be created

Acetate	Formate
AIEX-mix	HEPES
Bicine	MES
Bis-Tris	Methylpiperazine
Bis-Tris Propane	MOPS
Carbonate	Phosphate
CIEX-mix	Piperazine
Citrate	Succinic Acid
Diethanolamine	Triethanolamine
Ethanolamine	Tris

Fig 1. Example of chromatogram using CIEX-mix buffer recipe in combination with NaCl. Left side: pH is varied from 2 to 7 at the same time as NaCl is varied from 0 to 500 mM. Right side: pH 3 and NaCl is varied from 0 to 900 mM.