Dynamic system for simulation of physiological pH gradients for hydrogen carbonate buffers (pHysio-grad).

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ABSTRACT SUMMARY

The thermodynamic instability of the hydrogen carbonate buffers can be used for simulation of pH gradients of physiological ranges and dynamics without changing the ionic strength of the solution. For this purpose, acidification as well as degassing of the dissolution media require continuous and dynamic adjustment, which can be realized using the pHysio-grad® device.

INTRODUCTION

The hydrogen carbonate buffer is considered as the most biorelevant buffer system for the simulation of intestinal conditions and covers the physiological pH range of the luminal fluids form pH 5.5 up to about pH 8.4 [1, 2]. However, the pH value of a hydrogen carbonate buffer is a complex and dynamic interplay between the concentration of the hydrogen carbonate ions (HCO3⁻), concentration of carbonic acid (H2CO3), the amount of dissolved and solvated (CO2,aq, CO2•H2O), and the partial pressure of the CO2 (gas) above the solution [3]. The complex equilibrium between the different ions results in a thermodynamic instability of the solutions. In case of commonly used HCO3⁻ buffers, spontaneous loss of carbon dioxide (CO2) from the solution under dissolution test conditions results in an uncontrolled increase of the pH value. To date, many efforts were made to maintain the pH value of the HCO3⁻ buffers on the desired level by mineralization of the CO2 losses or quantitative substitution of the escaped CO2 by feeding the solution with the respective amount of CO2 gas using controllers such as the pHysio-stat® device.

Interestingly, the thermodynamic instability of the hydrogen carbonate buffers offers very advantageous opportunity to simulate the intraluminal pH changes of physiological ranges and dynamics. For this purpose, both processes, the acidification as well as the degassing of the dissolution media require continuous and dynamic adjustment. It is well know, that the acidification of the commonly used HCO3⁻ buffers is a rather fast process whereas the CO2 loss occurs relatively slow and spontaneously. However, the degassing process under dissolution test conditions can be accelerated and controlled by purging the solution with an inert gas such as N2. The pH changes of the HCO3⁻ media come along with displacement of the CO2 (gas) between the solution and the atmosphere above the solution. The electric load of the spectator cations such as K⁺, Na⁺ commonly used as hydrogen carbonate salts is compensated by HCO3⁻ in the case of acidification or OH⁻ resulting from water hydrolysis in the case of alkalinization. Therefore, the resulting total ion concentration and the ionic strength in the intraluminal pH range remain nearly constant.

The present work aimed at the development of a device that enables automatic pH monitoring and regulation as well as simulation of pH gradients within physiological pH range for dissolution tests performed with bicarbonate based buffers.

EXPERIMENTAL METHODS

pHysio-grad®: is a novel microprocessor-driven system which enables the adjustment and variation of the pH value in one dissolution vessel. The system is composed of a control unit, a pH electrode and proportional valves controlling the flow of CO2 and N2.
Test conditions: the tests were performed in the USP apparatus 2 operating at 100 rpm and 37.5°C and 1000 mL fill volume.

Dissolution medium: solution containing 1mMol/L NaHCO₃ was used. The initial pH value was adjusted to pH 5.5 by purging CO₂ and subsequently changed stepwise by purging CO₂ or N₂.

pH-measurement: The pH was monitored continuously using reference pH meter at a sampling rate of 0.34 Hz.

Conductivity-measurement: The conductivity was monitored continuously using reference conductometer at a sampling rate of 0.034 Hz.

Gases: N₂, CO₂ were introduced into the controller at a pressure of 1 bar and dosed into dissolution media via diffusor.

RESULTS AND DISCUSSION
The results of the suitability tests of the pHysio-grad® device for pH adjustment and simulation of pH gradients as well as the results of the conductivity measurements are given in Figure 1.

![Figure 1: Results of conductivity measurement (▲) and pH adjustment (●) of the model media using pHysio-grad® device. The arrows indicate the dosing of gases N₂ (nitrogen) and CO₂ (carbon dioxide).](image)

The data indicate that by purging the solution with N₂ the pH can be rapidly increased up to the desired level. The CO₂ can be rapidly removed from the solution which results in an increase in the pH value (~ 1.5 pH units/min). The pH of the solution can be also rapidly decreased by purging the solution with CO₂ (> 2 pH units/min). The conductivity of the solution during the experiment remains nearly constant suggesting that the total concentration of ions in the solution and thus, the ionic strength remains practically unchanged at different pH values. The test results confirm the suitability of the pHysio-grad® device for pH adjustment, monitoring and simulation of pH changes of model hydrogen carbonate media.

CONCLUSION
The resulting pH value of hydrogen carbonate buffers is a complex and dynamic interplay between the concentration of HCO₃⁻, the concentration of H₂CO₃, the amount of dissolved and solvated CO₂ as well as the CO₂ partial pressure above the solution. The thermodynamic instability of the hydrogen carbonate buffers offers very advantageous opportunity to simulate the intraluminal pH changes of physiological ranges and dynamics without changing the ionic strength of the solution. For this purpose both processes, the acidification as well as the degassing of the dissolution media require continuous and dynamic adjustment. This can be realized by using the pHysio-grad® system, which enables precise pH adjustment, monitoring and simulation of pH changes of model hydrogen carbonate media for dissolution testing.

REFERENCES