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Brief/Technical Note

An Automated System for Monitoring and Regulating the pH of Bicarbonate Buffers

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Abstract. The bicarbonate buffer is considered as the most biorelevant buffer system for the simulation of intestinal conditions. However, its use in dissolution testing of solid oral dosage forms is very limited. The reason for this is the thermodynamic instability of the solution containing hydrogen carbonate ions and carbonic acid. The spontaneous loss of carbon dioxide (CO_2) from the solution results in an uncontrolled increase of the pH. In order to maintain the pH on the desired level, either a CO_2 loss must be completely avoided or the escaped CO_2 has to be replaced by quantitative substitution, i.e. feeding the solution with the respective amount of gas, which re-acidifies the buffer after dissociation. The present work aimed at the development of a device enabling an automatic pH monitoring and regulation of hydrogen carbonate buffers during dissolution tests.

KEY WORDS: bicarbonate media; hydrogen carbonate buffer; modified release; physiological buffers; biorelevant dissolution.

INTRODUCTION

The pH of the luminal fluids in the intestines ranges from pH 5 up to 8.5 and depends on the GI segment and the prandial conditions (1,2). Contents of the small intestine are buffered mainly by bicarbonate ions, which are secreted by the pancreas and intestinal epithelial cells. Other ions and luminal constituents, such as bile salts, proteins, carbohydrates, and other food components also contribute to the final buffer capacity and the pH (3–6). However, as they play a minor role, the bicarbonate buffer is considered being the most biorelevant buffer system for the simulation of intestinal conditions (7,8). The carbonate buffer also reflects the ionic composition and the buffer capacity of small intestinal fluids (7). Therefore, this buffer system is essential for a realistic simulation of intestinal conditions, particularly, when it is aimed to predict drug release from solid oral dosage forms containing ionizable drugs or excipients. In numerous studies, it was demonstrated that the dissolution of such formulations can be significantly affected by the ionic composition and the concentration of ions in the dissolution media (9–11). Furthermore, it has been shown that bicarbonate buffers are more discriminative than compendial phosphate buffers and allow a better estimation of drug release from pH responsive formulations such as coated dosage forms intended to release the drug in the ileocecal region. It has also been demonstrated that bicarbonate buffers media enable a better prediction of *in vivo* disintegration times and drug release from products containing ionizable compounds (4,7,8,12–15).

The use of bicarbonate-based media in the routine analysis of the dissolution performance of solid oral dosage forms is very limited. If at all, Krebs buffer and Hanks buffer as well as modifications thereof are typically used as bicarbonate-buffered media (7,8). However, due to the thermodynamic instability of these solutions containing bicarbonate ions and carbonic acid, unpredictable pH rises due to the loss of CO_2 from the solution occur over time. Carbonic acid is a weak acid (pK_a~6.4), which is spontaneously formed by the dissolution of its anhydride, i.e., carbon dioxide (CO_2) in water (8,16). The pH of aqueous solutions of CO_2 ranges from pH 3.9 to 6.97 and is dependent on the partial pressure of CO_2 and the temperature of the fluid. In aqueous solutions of CO_2, bicarbonate (HCO_3^-) and carbonic acid (H_2CO_3) coexist in equilibrium as illustrated in Eq. 1. The composition of the solution within the physiological pH range can be calculated considering the equilibriae among the two different carbonate forms (H_2CO_3 and HCO_3^-), the hydration equilibrium between dissolved CO_2 and H_2CO_3 as well as the equilibrium among the dissolved CO_2 and the gaseous CO_2 (the bicarbonate equilibrium and dissociation):

$$CO_2\,(gas) \underset{K_{sol}}{\overset{K_{los}}{\longleftrightarrow}} CO_2\,(aq) + H_2O \overset{K_{hyd}}{\longleftrightarrow} H_2CO_3 \overset{K_{diss}}{\longleftrightarrow} H^+ + HCO_3^- \quad (1)$$

The K_{sol} expresses the solvation of CO_2 (gas) in the aqueous medium. According to Henry's law, at a given temperature

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the composition of a pure carbonic acid solution is determined solely by the partial pressure of the CO_2 purged into dissolution medium ($P(CO_2)$) and is as high as 29.76 atmmol^{-1}L^{-1} at a temperature of 25°C (Henry constant). For this reason, it can be assumed that the total concentration of solubilized carbon dioxide (CO_2 (aq)) can be expressed by Eq. 2 (solubility equilibrium of CO_2 (gas)).

$$[CO_2\ aq] = K_{sol}P(CO_2)$$

Under normal conditions (25°C, 1 bar), the equilibrium constants of carbonic acid are $10^{-2.2}$ for hydration 1 (K_{hyd}) and about $10^{-3.7}$ for dissociation (K_{diss}). The overall dissociation constant of carbonic acid (pK_a) into the bicarbonate ion ranges from $10^{-6.4}$ up to $10^{-6.0}s^{-1}$. The CO_2 loss from the solution due to spontaneous media degassing is expressed by the $K_{los,}$ which is dependent on physical parameters, such as temperature, agitation rate, volume/surface ratio of the dissolution media, and the gas exchange on the media surface. This indicates that various test parameters can have a significant impact on the composition of the test medium over the duration of the test. To keep the system in an equilibrium according to Eq. 1, as a result of the CO_2 an uncontrolled increase of the media pH due to protonation of bicarbonate ions can be observed. The buffer capacity of pure bicarbonate solutions is pH dependent. It is highest around the pK_a (~6.4) and does practically not exist below a pH of 5.5 (16). The presence of cations of strong electrolytes, such as alkaline metals increases the concentration of bicarbonate ions in the solution. However, for the pH adjustment in the pH range below the hydrolysis pH of bicarbonate salts (which is about pH 8.4), additional amounts of carbonic acid have to be introduced into the solution which increases the capacity of the HCO_3/H_2CO_3 buffer system.

As a result of the abovementioned phenomena, the pH of bicarbonate solutions can be maintained at predetermined levels by either preventing CO_2 loss or replacing evaporated CO_2. CO_2 loss can be minimized by the use of seal devices for dissolution testers or by covering aqueous dissolution media with organic layers such as paraffin to prevent degassing of carbonate-based dissolution media during the experiment (8). The pH can also be maintained by replacing the escaped CO_2 via feeding an equal amount of CO_2 (gas) into the solution. After dissolution and subsequent dissociation, the introduced CO_2 re-acidifies the buffer. A mixture of 5% CO_2 in N_2 is suitable for this purpose and provides a partial pressure of CO_2 that enables to maintain the pH value at the level of 7.4 (8). This mixture is added to the dissolution medium until the desired pH value is achieved. However, to date the intensity of the continuous or pulsatile gas flow has typically to be adjusted manually (7,8,14). Overall, current methods applied for the pH stabilization of aqueous media are strictly related to particular test conditions and often not reproducible. Moreover, their utilization is time consuming, and they are hardly applicable for quality control tests.

The objective of the present work was the development of a device enabling an automated pH monitoring and regulation of bicarbonate buffers during their application in dissolution experiments. The device should provide a reproducible adjustment of the pH of bicarbonate media within the pharmacopoeial tolerance range of ±0.05 pH units and should be characterized by high regulation dynamics, a simple construction, and a high robustness. Furthermore, the device should be a stand-alone system and should be compatible with commonly applied compendial dissolution test setups of different origin.

MATERIALS AND METHODS

Test Device

The developed system called "pHysio-stat" is an automated device represented by a novel microprocessor-driven system which enables the adjustment of the media pH in a single dissolution vessel. The system is composed of a pH electrode (InLab Science expert Pro, Mettler Toledo, Greifensee, Switzerland), a gas diffuser (1 μm PES filter, ERWEKA GmbH, Heusenstamm, Germany), a digital microcontroller and a proportional valve system (SCG202A50124C, Emerson, Hamburg, Germany). The system is driven by custom-made software based on an AVR-GCC open source platform (ARDUINO, Italy). The software controls the pH adjustment and enables the setting of trigger values as well as a regulation hysteresis. In the system operation mode, the pH electrode and the gas diffuser remain permanently immersed (about 35 mm deep) in the dissolution medium. Over the entire experiment, the potential of the electrode is measured and digitalized by the controller at a rate of 1 Hz. The digital signal is then processed to be instantly applicable for the regulation of the proportional valve that adjusts the amount of CO_2 introduced into the dissolution medium via the diffuser. The diffuser is connected to the device with a 4-mm polypropylene tubing of 1.2 m length. The device is a stand-alone system, which can be used in various dissolution test devices. A schematic and photographic representation of the pHysio-stat system is given in Fig. 1.

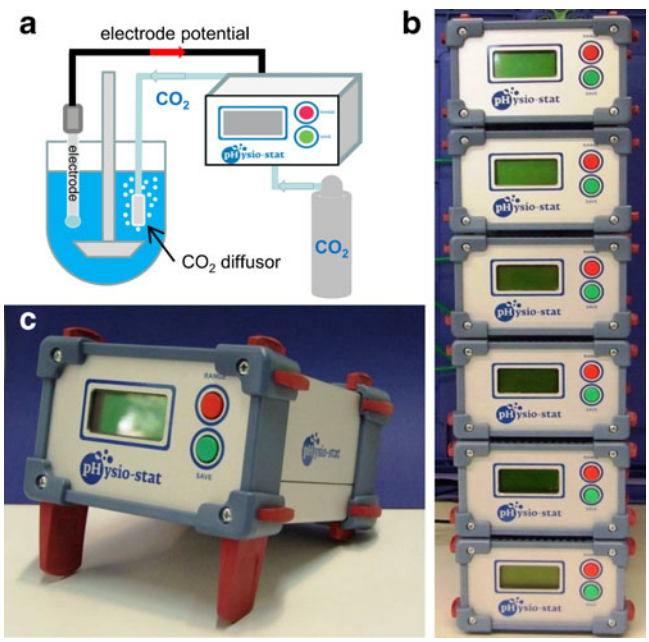

Fig. 1. Photographic and schematic representation of the pHysio-stat device

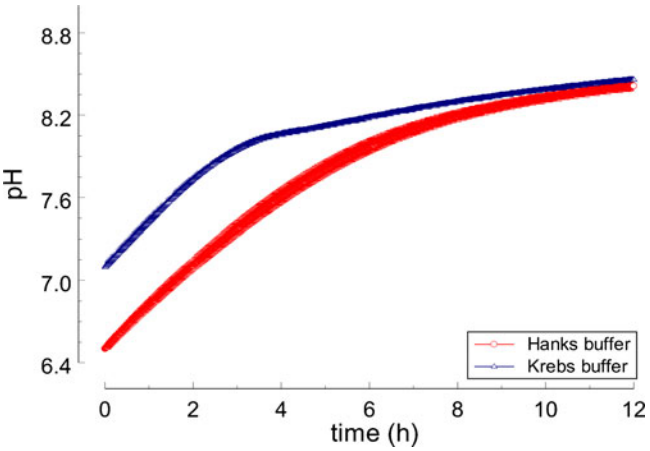

Fig. 2. Mean (n=3) pH profiles of Hanks and Krebs buffers during dissolution test in the USP Apparatus II operating at 100 rpm, 1,000 mL filling volume, and 37.0±0.05°C without pH adjustment. The standard deviation is indicated by the *error bars*; the sampling frequency was 0.2 Hz

Test Objectives

The tests aimed at the investigation of the precision of the pH adjustment over short and long time intervals (4 and 12 h). Moreover, the effectiveness and dynamics of the pH adjustment were investigated. For this purpose, every 5 min 150 µL of a 0.5 mol/L NaOH solution were added to the dissolution media and the pH was measured continuously over a period of 1 h.

Test Conditions

The novel pHysio-stat device was evaluated using USP Apparatus II (Paddle) operating at 50 and 100 rpm and 37°C (Erweka DT7R, Erweka GmbH, Heusenstamm, Germany). Before investigating the applicability of the pHysio-stat device, an initial set of experiments was performed where the pH rise due to spontaneous loss of CO_2 from the dissolution media was monitored in dissolution tests performed under the proposed screening (test) conditions but without any pH adjustment. For this purpose, the dissolution vessels were equipped with a polyacrylate cap covering >98% of the vessel surface in order to reduce the medium losses due to evaporation and gas exchange at the media surface.

Dissolution Media

Dissolution media used in our study comprised Hanks buffer with a pH of 6.5, 6.8, or 7.4, Krebs buffer of pH 7.4 and a $NaHCO_3$ solution (0.5 g/L) of pH 7.4 as an example of a medium representing very low buffer capacity and electrolyte concentration (7,8). The media volume was 1,000 mL in all experiments. The pH of the dissolution media was adjusted and measured manually (SevenGo Multi equipped with InLab Expert Pro pH electrode, Mettler Toledo, Greifensee, Switzerland) immediately prior to analysis by manual dosing of 10% CO_2/N_2 gas mixture or if necessary small amounts (<0.01 mM/L) of NaOH aq or HCl.

Titer Gas

A mixture of 10% (vol/vol) of CO_2 in N_2 (Linde AG, Greifswald, Germany) was used as a titer gas. The gas was introduced into the controller at two different pressures; 0.5 and 1 bar IR-1000F1 pressure controller, (SMC, Egelsbach, Germany) and dosed into dissolution media via the diffuser.

pH Measurement

During the experiments, the pH of the dissolution media was monitored continuously with a sampling frequency of 0.2 Hz using a calibrated multichannel pH meter (EAInstruments Limited, MCC-SYST-6B, Middlesex, UK) equipped with reference electrodes (InLab Expert Pro, Mettler Toledo, Greifensee, Switzerland). The data were recorded by a personal computer and processed using commercial software (Axum 5.0, Adept Scientific, Letchworth Garden City, UK).

RESULTS

The test results are presented in Figs. 2, 3, and 4. In the dissolution experiments performed without pH adjustment, the pH increase amounted to approximately 0.5 pH units/h in Hanks buffer and about 0.6 pH units/h in Krebs buffer. Independent of the applied buffer system, a final pH of approximately pH 8.4 was reached (Fig. 2). In contrast, results obtained from the experiments where the pH-stat device was applied clearly indicate that independent of the dissolution medium, the pH-stat system enabled a precise pH adjustment with a maximum deviation of ±0.05 pH units (Figs. 3 and 4).

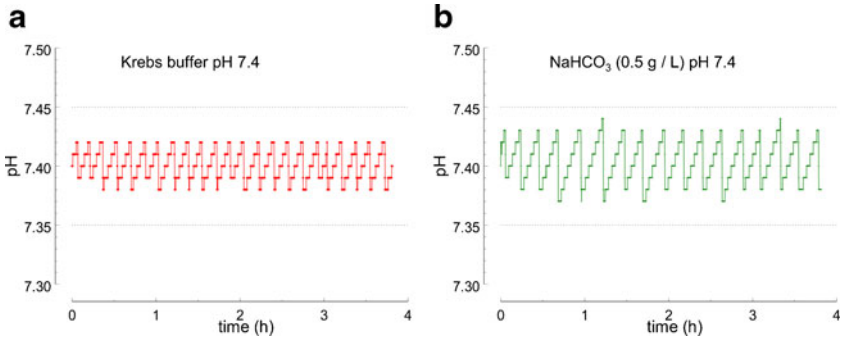

Fig. 3. Short-time accuracy; set point, pH 7.4; tolerance range, ±0.05 pH; individual values; USP Apparatus II operating at 100 rpm, Krebs Buffer (**a**) and $NaHCO_3$ solution (**b**) of pH 7.4, 1,000 mL filling volume, 37.0±0.05°C, operating pressure 1 bar, and a sampling frequency of 0.2 Hz

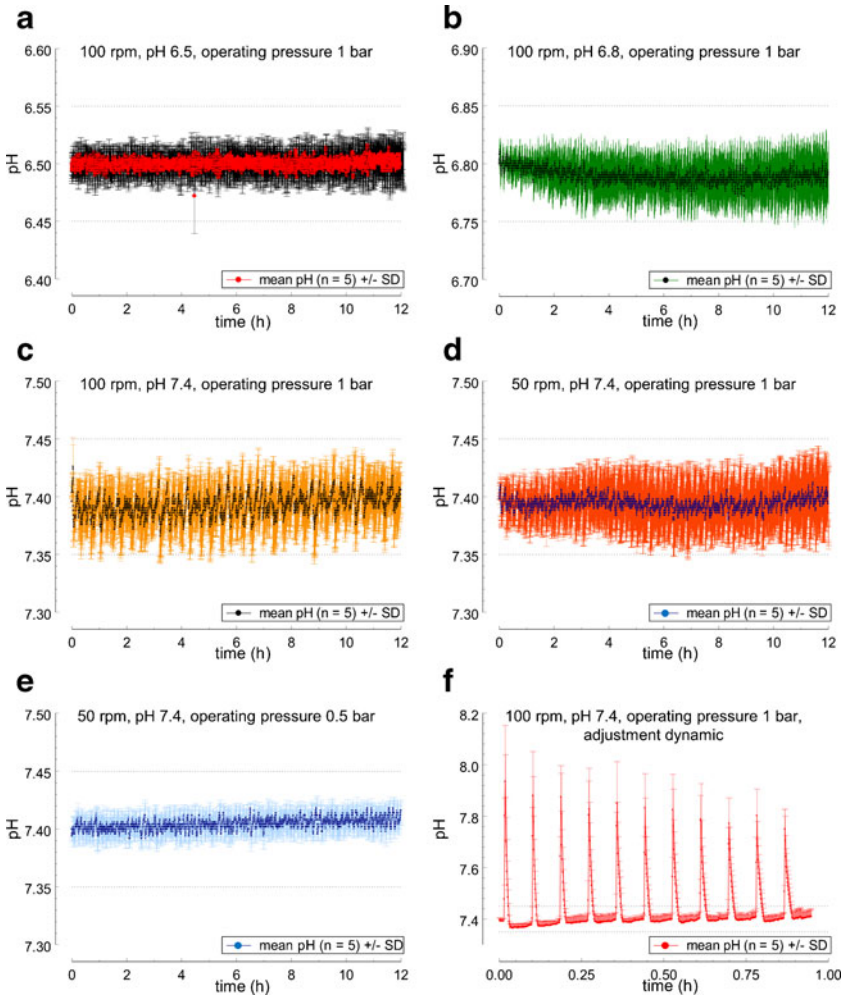

Fig. 4. Accuracy (**a**–**e**) and dynamics (**f**) of the pH adjustment, at various set points and tolerance range of pH±0.05, mean of n=5 devices±SD, USP Apparatus II, Hanks and Krebs buffer, 1,000 mL, 37.0±0.05°C, sampling frequency of 0.2 Hz. The stirring rates, operating pressure, and set pH are given in the *insets*

This was true for both common bicarbonate media like Krebs and Hanks buffer as well as a 0.5 g/L sodium bicarbonate solution of very low buffer capacity. Overall, pH fluctuations became higher with increasing pH (Fig. 4a–c). The mean root square of the pH change as well as the mean root square error of the pH adjustment for each test condition presented in Fig. 4a–e are given in Table I. The pH adjustment was only slightly affected by stirring rates of 50 or 100 rpm (Fig. 4c, d), but was apparently affected by the pressure of the introduced titer gas (Fig. 4d, e; Table I). By reducing the operating pressure from 1 to 0.5 bar the precision of the regulation process was significantly improved and concomitantly, as indicated by the decrease in the mean square root pH change as well as the mean square root error, the pH fluctuations could be minimized.

In the experiments where 150 μL of a 0.5 mol/L NaOH solution (75 μM NaOH) was added to the media at predetermined time points, the pH adjustment to the desired value was completed within 45–60 s after addition of the NaOH solution (Fig. 4f). The initial pH increase (ΔpH) measured immediately after NaOH addition was systematically decreasing from

Table I. The Mean Root Square of the pH Change as well as the Mean Root Square Error of the pH Adjustment Calculated for Different Test Conditions

Test conditions	pH 6.5, 100 rpm, and o.p. 1 bar	pH 6.8, 100 rpm, and o.p. 1 bar	pH 7.4, 100 rpm, and o.p. 1 bar	pH 7.4, 50 rpm, and o.p. 1 bar	pH 7.4, 100 rpm, and o.p. 0.5 bar
Mean (8,640 data points) square root pH change	0.00415	0.00905	0.01203	0.00812	0.00647
Mean (n=8,640) square root error	0.01310	0.02101	0.02105	0.02420	0.00911

o.p. operating pressure

approximately 0.7 pH units after the first NaOH addition to about 0.4 pH units after the 11th addition. This was most likely due to the increase in buffer capacity caused by the addition of sodium ions to the existing buffer system. In summary, under the applied test conditions the functionality of the novel pH-stat system was characterized by an excellent short- and long-term stability as well as by a highly dynamic regulation process.

DISCUSSION

Hanks and Krebs balanced salt solutions are intended to realistically mimic the ionic composition of distal small intestinal fluids. However, both media are characterized by very low buffer capacities ranging from 1 $mML^{-1}\Delta pH^{-1}$ for the Hanks buffer to 5.45 $mML^{-1}pH^{-1}$ for the Krebs buffer at pH 7.4. Recently, the composition of Hanks buffer was modified to match the buffer capacity of the ileal fluids, which is about 6.4 $mML^{-1}pH\ unit^{-1}$ (7). However, due to their very low buffer capacities, the pH adjustment of bicarbonate media is difficult to achieve under routine dissolution test conditions. It can be either realized by reducing the CO_2 loss or by adequately substituting the evaporated CO_2 (7,8,14). The CO_2 loss can be at least partly compensated by purging the bicarbonate buffers with gas mixtures of an appropriate partial pressure of CO_2. Such purging accounts for the bicarbonate equilibrium in the buffer solution and thus enables to maintain the medium pH at the desired level. Typically, mixtures of 5% (vol/vol) CO_2 and N_2 are used for this purpose (8). The main drawback of this approach is the limited pH range that can be controlled by using a single particular gas mixture. Moreover, the control of pH values within the desired tolerance range often implies the adjustment of the gas flow, which with a view to the duration of a typical dissolution experiment, particularly when testing controlled release formulations, requires manual intervention/control over rather long time intervals. In current dissolution test methods, pH checks and adjustments are routinely performed every 15 to 30 min and need to be performed separately for each single dissolution vessel. This results in a work-intensive and time-consuming procedure which due to the frequent intervention can also result in a reduced precision of the methodology. Overall, such methodologies are rather inefficient and not applicable for routine experiments. Interestingly, the main drawbacks related to the use of bicarbonate buffers in dissolution tests can be overcome by the use of a device for automated pH monitoring and adjustment.

In the present work, we present an automated controller for monitoring and adjusting the pH of bicarbonate media. The new pHysio-stat device enables an automated dosing of CO_2 gas and mixtures thereof into bicarbonate-based dissolution media in order to achieve and maintain the pH at a desired level. The pH value can be adjusted and controlled within the pharmacopoeial tolerance range of ±0.05 pH units over time intervals of more than 12 h (up to 3 days, data not shown) with an adjustment rate of 1 Hz. Furthermore, the pHysio-stat system can use gas mixtures of different CO_2 partial pressures or even pure CO_2 as titer gas. It enables the pH adjustment in the pH range of pH 5.5 to 8.4 covering the intraluminal pH variability of intestinal fluids in both the fasted and fed state. Considering the dissociation constants of CO_2 (Eq. 1), it is likely that the pH of the bicarbonate buffer solutions can be realistically decreased to the level of pH 5.5 or even lower. This can be realized by purging the dissolution media with gas mixtures of high partial pressure of CO_2 or even pure CO_2. In the present work, we observed that the pH adjustment showed a higher precision at lower pH values. This circumstance is related to the dissociation of carbonic acid and the buffer capacity of the bicarbonate media, which is highest close to the pK_a of carbonic acid which is about 6.4. In the tests performed at pH 7.4, somewhat higher fluctuations with values of ΔpH ranging from −0.03 up to −0.04 pH units were noticed immediately after purging. The observed "overregulation" is related to the slower reaction rate of CO_2 (gas) dissolution and dissociation as well as to test parameters, such as the amount of titer gas remaining in the tubing that connects the proportional valve with the diffuser, the electrode response and the mixing conditions in the USP Apparatus II (16–19). However, both the dynamics and the precision of the pH adjustment can be regulated flexibly by appropriately setting the pressure of the titer gas introduced into the device as well as by the adjusting the device settings such as trigger pH difference, regulation range, and hysteresis. In order to maintain the continuity of the pH monitoring and adjustment processes, both the pH electrode and the diffuser have to be continuously immersed in the dissolution medium during the test. Apparently, the placement of the pH electrode and the gas diffuser in the upper part of the dissolution vessel (~3 cm below media surface) do not influence the hydrodynamic conditions at the bottom part of the vessel, where the dosage form is supposed to stay during the dissolution experiment in the paddle apparatus (20,21). However, the changes in the hydrodynamics caused by the gas diffuser as well as the electrode were not studied in the present work and require further investigations.

CONCLUSIONS

The novel pHysio-stat system can precisely monitor and adjust the pH value of commonly used bicarbonate buffers and is characterized by sufficient robustness, good performance stability as well as high dynamics of the pH adjustment processes. These unique features make the pHysio-stat system particularly useful for routine applications in dissolution tests in which bicarbonate based buffers are utilized.

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