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A Simple Pharmacokinetic Model Based on Mean Residence Times to Predict Furosemide Exposure after Oral Doses

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ABSTRACT

Oral bioavailability of furosemide is low (around 70%) even if a solution of 40 mg is given to healthy subjects. Sustained release formulations rendered even lower absorption of drug (one half of immediate release formulations). First pass metabolism in the stomach, slow dissolution of solid formulations in acidic media, and a narrow absorption window in the upper portion of the small intestine, are the main causes for such low bioavailability. All these processes were considered in a pharmacokinetic model in order to predict furosemide plasma concentrations after oral administration. Mean times (inverse of first order rate constants) were used instead of rate constants because of their easier handling and understandability. Pharmacokinetic values for the model were obtained from the literature. Mean dissolution times (MDT) for different Uruguayan formulations were obtained experimentally by assaying them at pH 4.6 with USP-2 and USP-4 apparatus, and at variable pHs (1.6 to 6.5) with biorelevant media in USP-4. Based on the *in vitro* obtained MDT, furosemide plasma exposures have been simulated accordingly with this simple pharmacokinetic model in order to explore different bioequivalence scenarios.

INTRODUCTION

Furosemide is a loop diuretic that is normally used in the treatment of edematous states associated with cardiac, renal, hepatic failure and in the treatment of uncontrolled hypertension with abnormal renal function. Its oral bioavailability is low (67%) even if a solution of 40 mg is given to healthy male subjects[1]. In a study carried out with women and men[2], absolute bioavailability under fasting state was reduced to 51% revealing a sex-related influence on the oral absorption of furosemide. Sustained release formulations rendered even lower drug absorption[3, 4]. Switching from intravenous bolus to oral slow release forms more than three quarters of the administered dose are not absorbed. Therefore, drug dissolution might play a significant role in furosemide absorption as it was previously reported[5, 6].

However, not only dissolution seems to condition the amount of furosemide absorbed. Some authors[1] reported that an oral solution has similar bioavailability to tablets. Hence, an important percentage of the dose (about 30%) does not cross the intestinal barrier in order to enter into the body. Several hypotheses were proposed to explain this fact. One of them, the most recently studied, is the role of efflux transporters at the enterocyte moving the molecules back to the intestinal lumen[7, 8]. The most ancient and best known one is based on the low lipophilicity of furosemide. Its high ionization at intestinal pHs determines a narrow absorption window in the upper portion of the small intestine[9, 10]. The increase of both pH and the expression of efflux transporters along the small intestine[11, 12] would reduce the ability of furosemide to cross the intestinal epithelium a short distance ahead from the pylorus.

Interestingly, the linear pharmacokinetic response observed after increasing oral solution doses of furosemide[13] was not reproduced when plain tablets were given[14, 3]. Oral solution doses of 20, 40 and 80 mg assayed in healthy men rendered unchanged urinary furosemide recoveries (twenty-four-hour urine) of 9.62, 16.7 and 32 mg respectively, while 16.2 mg and 17.8 mg were recovered after the intake of 40-mg and 60-mg tablets (Furix ®) respectively when they were assayed in two different groups of healthy subjects of both sexes.

Causes for the lack of dose linearity with oral solid forms have not been explored in the literature. The time to dissolve the granules of furosemide coming from solid dosage forms should be longer than the time to dissolve gastric precipitated microparticles coming from

solutions once they enter into the duodenum. Then, residence time of solid particles at the absorption window is critical to accomplish the total absorption of furosemide. A higher intestinal dispersion of solid microparticles generated after the ingestion of solutions allows the homogenization of the luminal compartment, and then, the different processes: dissolution, disappearance from the site of absorption towards the lower portion of the intestine, and permeation through the membrane, could be first order processes, and the fraction of dose absorbed could remain constant. Conversely, the non-dissolved granules coming from solid dosage forms do not allow such compartment homogenization and then, the kinetics of dissolution and mass movements throughout the gut would change with doses in a nonlinear manner. In fact, this phenomenon constrained the efficiency of slow-release formulations[15], leading to a progressive loss of bioavailability while they are moving along the intestine. In order to solve this inconvenience, the drug should be delivered to the duodenum already dissolved. This was the reason why gastroretentive delivery systems have been promoted to prolong furosemide absorption and to maintain the bioavailability of rapidly dissolving formulations[10].

Immediate release dosage forms reduced furosemide bioavailability following postprandial administration[2, 16]. Lower area under the plasma concentration time curves (AUC), lower unchanged drug urinary recovering (U), lower maximum plasma concentration (C_{MAX}), and delayed time to peak (T_{MAX}) were the most relevant observed changes when both tablets and oral solution were given with food[2]. Pre-systemic gastric metabolism suggested by Lee and Chiou (1983)[17] could partially explain this decrease of bioavailability. The increase of gastric pH due to food ingestion could increase the ionization of furosemide at the intestine in male subjects. This can be observed from data published by Hammarlund et al. (1984)[2] where immediate release tablet reduced furosemide bioavailability drastically in some subjects, presumably men, when food was coadministered. It should be borne in mind that women have a higher gastric pH than men[18, 19], and consequently, the impact of pH increase on furosemide bioavailability after food intake could be lower.

Figure 1 shows a pharmacokinetic model that takes into consideration the main processes, mentioned above, that oppose to the entrance of furosemide into the body when it is orally administered either by solutions (red numbers) or by immediate release forms (blue numbers). Times in the arrows represent the inverse of the involved first order rate constant. This way of

referring first order rate constants retrieves the concept of mean residence times for such processes, making kinetic constants more understandable. A two-compartment model was assumed in order to describe furosemide disposition. The disposition pharmacokinetic parameters were adapted from those of Hammarlund et al. (1984)[2].

Based on the information given by Lee and Chiou (1983) [17] a mean time of 280 min could be assessed for the metabolic first pass process taking place at the stomach (compartment G). Since the fraction of furosemide dissolved in the stomach is minimal after the intake of solid oral forms, a twofold higher value (560 min) for such formulations was assumed. Mean gastric emptying times of 15 minutes for solution and 30 minutes for immediate release solid formulation, under fasting conditions, seemed to be realistic according to the work of McNamara et al. (1987)[6].Then, competitions at the stomach between drug metabolism and drug passage to the intestine will just reduce the bioavailability up to 0.95 for tablet and solution.

Once the solution enters into the intestinal lumen, molecules become rapidly available to permeate the mucosa even though the rate of such transfer would not be very fast due to ionization. In order to satisfy the bioavailability of 70% for oral solutions, mean permeation time of 50 min and mean no-permeation time of 200 min, were then assumed. Considering that first order rate constants are the inverse of mean times, the permeation fraction can be calculated as the ratio between the permeation rate constant and the sum of both permeation and no-permeation rate constants [1/50]/[1/200+1/50].

McNamara et al. (1987) [6] and Prasad et al. (1982)[5] developed good correlations between *in vitro* and *in vivo* parameters (IVIV) when dissolution testing was performed in USP-2 apparatus, at a pH of 4.6, in 900 mL of buffer acetate, with 50 rpm of stirring. Interestingly, *in vitro* and *in vivo* mean dissolution times[6] could also be linearly correlated.

Mean times of 20 min[6] for dissolution at the gastrointestinal tract (for 40-mg Lasix® tablets), and 50 min for pre-systemic elimination process getting the drug away from the absorption route, were combined at the dissolution compartment of the model. Thus, both the mean absorption time (84 min) and the bioavailability (51%) reported by Hammarlund et al. (1984) [2] were satisfactorily accomplished.

Model in Figure 1 allowed us to reproduce, in the group of subjects participating in the study of Hammarlund et al. (1984)[2], the McNamara et al. (1987)'s plasma profiles[6] and cumulative urinary recoveries of the assayed oral formulations just by introducing their respective *in vitro* mean dissolution times. Parameters included in the model only apply for furosemide administration in fasting conditions. For postprandial administrations other parameters, such as an extension in the gastric emptying time, an increase of the permeation time, and a decrease in the non-permeation time, should be taken into account.

The aim of our study was to forecast the *in vivo* performance of different Uruguayan formulations using the proposed model.

MATERIALS AND METHODS

The analyzed products where four brands marketed in Uruguay as immediate release tablets containing 40 mg of furosemide: Furosemide EFA(Antia Moll, batch 028, expiration date Jan/2019), Furosemide FU(FarmacoUruguayo, batch 167, expiration date Jul/2016), Fischermide® (Celsius Laboratory, batch 33163, expiration date Jul/2019) and Lasix® (Sanofi Aventis, batch 1D034M, expiration date May/2017).

Three *in vitro* dissolution procedures were carried out: 1) with the USP-2 apparatus at pH 4.6; 2) with the USP-4 apparatus at pH 4.6, and 3) with the USP-4 following sequential biorelevant media changing the pH from 1.6 to 6.5.

USP-2 pH 4.6 *in vitro* dissolution assay

Six units of each product were assayed in Distek® dissolution system 2100C equipment, configured with a model 89092EO Agilent peristaltic pump, and coupled with spectrophotometer Agilent 8453. A software ChemStation® CPU (Agilent) controlled all the dissolution testing. Dissolution was performed in medium acetate buffer pH 4.6, at 37 ± 0.5 ºC, volume 900 mL; stirring speed 50 rpm, sampling times: 5, 10, 15, 20, 30, 40, 60, 90 and 120 minutes.

Quantification of Furosemide was carried out by UV-absorption at 277 nm wavelength.

USP-4 pH 4.6 *in vitro* dissolution assay

Three units of each product were assayed using CE 7 smart USP-4 flow through dissolution system (Sotax). The assay was carried out in medium acetate buffer pH 4.6 at 37±0.5 ºC. Flow rate was set at 16 mL/min in order to allow the formulation to be in contact through 60 minutes with approximately the same volume of solvent (960 mL) as in the USP-2 assay. Samples were collected every 15 minutes during 180 min.

Quantification of Furosemide was carried out by UV-absorption at 277 nm wavelength, in spectrophotometer UV-Vis Thermo Spectronic model Helios Gamma, Thermo Scientific, UK.

USP-4 pH 1.6-to-6.5 *in vitro* dissolution assay with biorelevant media

Similarly, but only for Lasix and Furosemide EFA, dissolution studies were carried out in Fasted State Simulated Gastric Fluid (FaSSGF pH 1.6) for 60 min at a flow rate of 8 mL/min with sequential change to Fasted State Simulated Intestinal Fluid version 2 (FaSSIF-V2 pH 6.5) at a flow rate of 4 mL/min for 90 min. These conditions were set in order to simulate the *in vivo* dissolution of both formulations following a fasting administration[20, 21]. Composition of FaSSGF and FaSSIF-V2 can be seen in Table 1.

Mean *in vitro* dissolution times

Mean dissolution time (MDT) was calculated for each formulation by the quotient between the area under the first moment of the non-dissolved amount (ND) curve and the area under the non-dissolved amount versus time curve (AUMND and AUND respectively). AUND was estimated using the trapezoidal rule from zero to the last dissolution time. Extrapolation to infinite was done by adding the last ND divided by the first order dissolution rate constant (k_{dis}) term. Similarly, the trapezoidal rule was used to estimate the area under the non-dissolved amount multiplied by time (ND*t) versus time (t) curve (AUMND) from zero to the last dissolution time, and extrapolation to infinite was done by adding the last ND*t divided by k_{dis} and the last ND divided by k_{dis}^2 terms.

Simulation of furosemide plasma concentration

The differential equation system defining the model shown in Figure 1 was solved for the compartment 1 yielding a concentration-time function [C(t)] with five exponential terms. All mean times shown in the model were inverted in order to obtain first order rate micro-constants.

All black numbers and blue numbers shown in the model, including the corresponding MDT, characterized the pharmacokinetics of each assayed formulation. C[t] functions were interpolated at different times to display the corresponding profile of plasma concentrations. Calculations were made with Microsoft Excel.

RESULTS AND DISCUSSION

Figures 2, 3 and 4 show the dissolution profiles of formulations according to USP-2 pH 4.6, USP-4 pH 4.6, and USP-4 with biorelevant media assays, respectively. The obtained MDTs for Furosemide EFA, Furosemide FU, Fischermide® and Lasix® using USP-2 method were 10, 23, 26 and 87 min, respectively. Using USP-4 method, the corresponding MDTs were 32, 48, 51 and 81 min. Under sequential biorelevant media in USP-4, Furosemide EFA presented a MDT of 19 min while Lasix® presented a value of 14 min. To calculate these MDTs no lag-times were assumed for the assays carried out at a pH 4.6, while a lag-time of 60 min (elapsed time at gastric pH) was considered in the USP-4 with variable biorelevant media testing. The rationale for this approach is that, according to the model, formulations would be able to be absorbed *in vivo* once they entered to the intestine. As it can be seen (Figures 2 and 3), differences in MDT between the four formulations were reduced under USP-4 condition, even though their relative order was maintained. Even closer would be their dissolution profiles if they were tested in USP-4 following sequential biorelevant media, as it could be inferred from results obtained with Furosemide EFA and Lasix® (Figure 4).

Figure 5 displays different couples of Furosemide EFA and Lasix® plasma concentration-time profiles depending on the MDTs used for simulations.

Regarding the *in vivo – in vitro* correlation when dissolutions were tested at pH 4.6[6], Furosemide EFA might be assessed as bioinequivalent, with higher bioavailability than Lasix®. These formulations showed the greatest difference in the *in vitro* dissolution performance in both apparatus. However, when formulations were tested in biorelevant media with USP-4 under conditions that simulate the transit from gastric to intestinal fluids (Figure 4), the model generated furosemide plasma profiles of both products are close and they could be assessed as bioequivalent.

A further bioequivalence study will be carried out in order to confirm the presumptions assumed, and hence to rely on both the predictive dissolution method and on the pharmacokinetic model for furosemide absorption. Regarding the model predictability, different bioequivalence trials should be carried out in order to validate it, since gastric emptying and gastric and intestinal pHs might change depending on whether men or women are enrolled, or whether fasting or fed conditions are considered.

The advantage of using this simple pharmacokinetic model is that mean dissolution times could be easily switched from the *in vitro* to the *in vivo* scenario, and mean residence times at each site of the gastrointestinal tract could be adapted considering the sex of individual and the experimental conditions of drug product administration.

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FIGURES

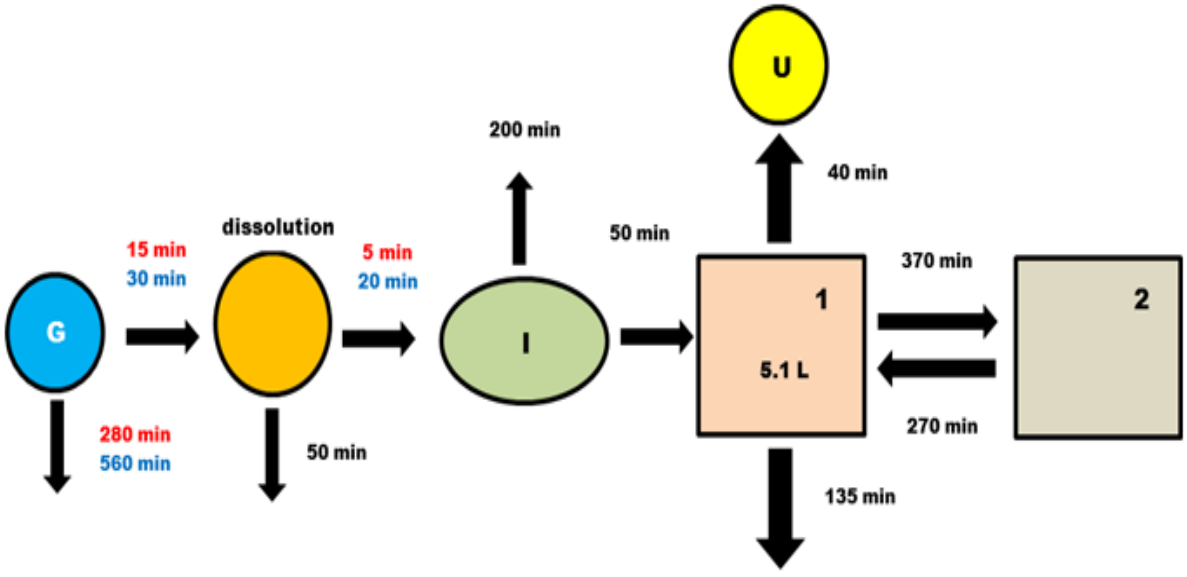

Figure 1: Pharmacokinetic model based on mean times to describe the absorption and disposition of furosemide

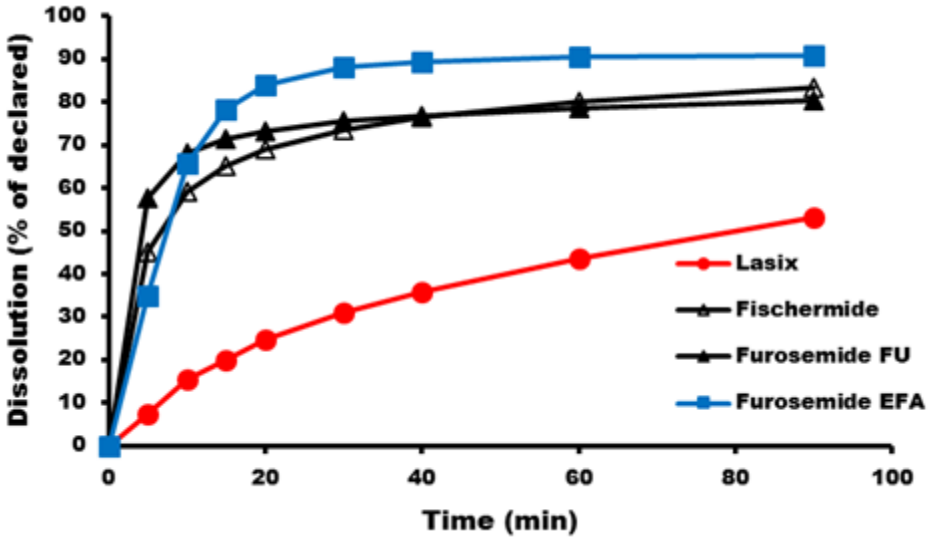

Figure 2: *In vitro* dissolution profiles of four furosemide oral solid formulations marketed in Uruguay using USP apparatus 2 method at pH 4.6

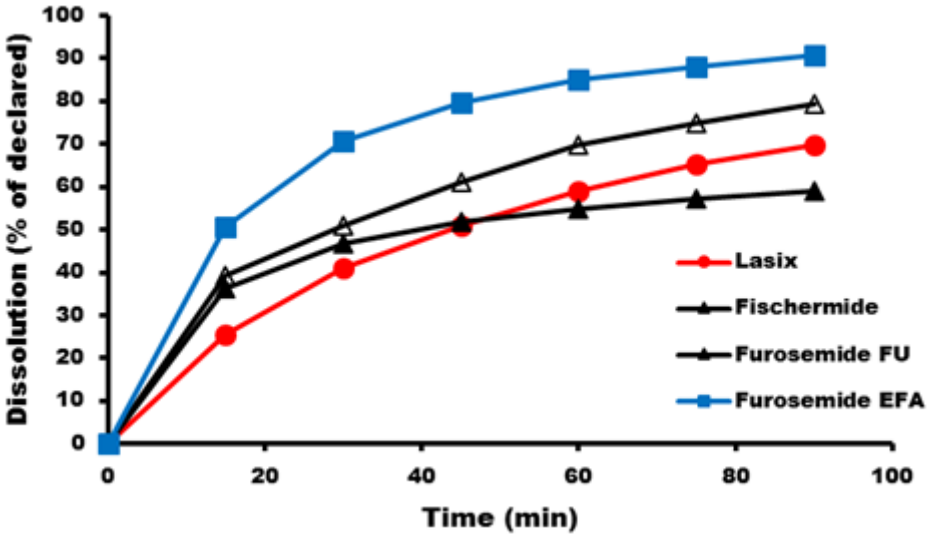

Figure 3: *In vitro* dissolution profiles of four furosemide oral solid formulations marketed in Uruguay using USP apparatus 4 and fixed pH 4.6 dissolution medium

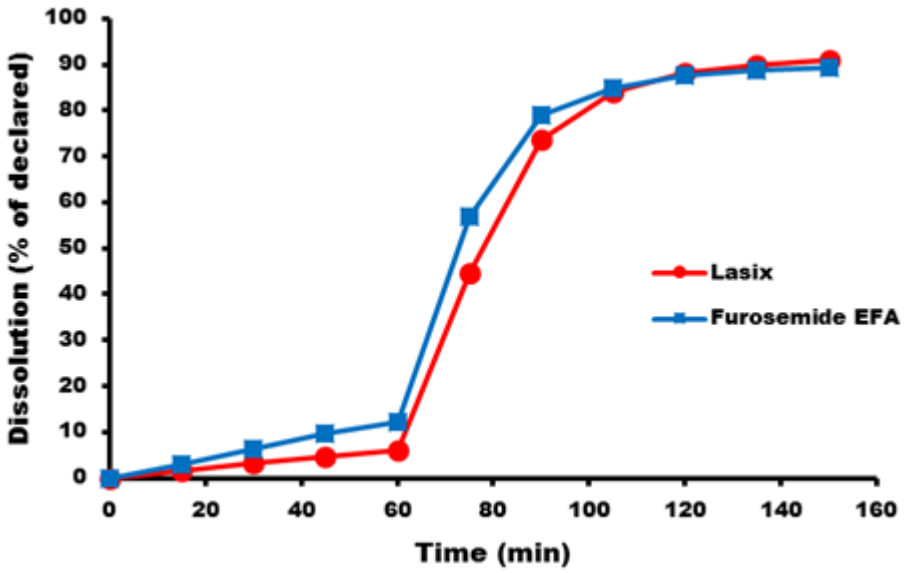

Figure 4: *In vitro* dissolution profiles of two furosemide oral solid formulations marketed in Uruguay using USP apparatus 4 and variable dissolution media, passing from FaSSGF pH 1.6 to FaSSIF-V2 pH 6.5

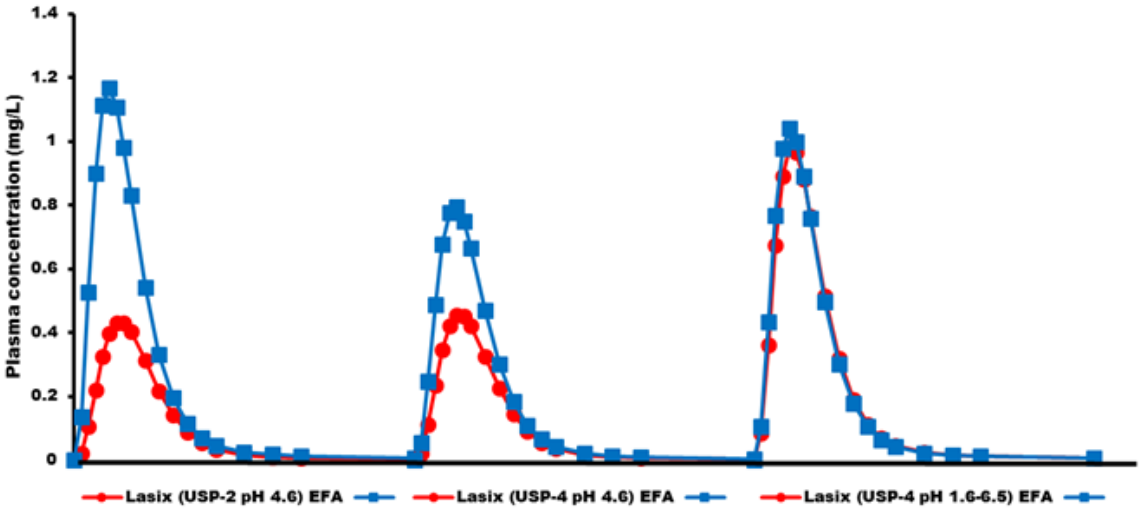

Figure 5: Simulated plasma concentration-time curves of two furosemide oral solid formulations marketed in Uruguay (Lasix® and Furosemide EFA) based on the mean dissolution times obtained from different *in vitro* dissolution methods

Table 1. Composition of biorelevant dissolution media

Component	FaSSGF	FaSSIF-V2
Sodium taurocholate (µM)	80	3000
Lecithin (µM)	20	200
Pepsin ($mg.mL^{-1}$)	0.1	-
Maleic acid (mM)	-	19.12
Sodium hydroxide (mM)	-	34.80
Sodium chloride (mM)	34.20	68.62
Hydrochloric acid	qs pH 1.6	-
pH	1.6	6.5
Osmolality ($mOsm.Kg^{-1}$)	120.7±2.5	180±10
Buffer capacity ($mmol.L^{-1}$.ΔpH^{-1})	-	10