Matching Large-Scale pCO_2 in Scale-Down Models: A Practical Manuscript

Abstract

Large-scale mammalian cell culture often runs at higher dissolved CO_2 partial pressure (pCO_2) than bench or pilot scale due to geometry-driven mass transfer and gas holdup differences. Failing to reproduce large-scale pCO_2 in a scale-down model (SDM) can bias pH control, buffer utilization, lactate/pyridine nucleotide metabolism, product quality, and CQAs. This manuscript unifies (i) a semi-empirical bicarbonate–CO_2–lactate acid–base balance to compute the equilibrium dissolved CO_2 required at a given pH setpoint and buffer state and (ii) a headspace overlay blending model to set the overlay %CO_2 (and/or flow) that attains a target gas-phase pCO_2 (e.g., 100 mmHg) observed at commercial scale. The result is an SDM recipe you can use directly in Excel or code to match large-scale pCO_2 while maintaining the intended pH and buffer equivalents.

1. Background and Problem Statement

At production scale, bioreactors frequently exhibit dissolved $pCO_2 \approx$ 80–120 mmHg because of increased gas holdup, longer bubble residence time, deeper headspace, and different gas-liquid interfacial dynamics. In contrast, lab-scale vessels with high sweep and efficient stripping often stabilize around 40–60 mmHg unless enriched. When pCO_2 is not matched, SDMs underestimate acid load from carbonic acid, overestimate apparent base capacity, and may misrepresent lactate dynamics and glycosylation outcomes.

Goal: Provide a reproducible method to (1) compute the required dissolved CO_2 for a target pH given lactate and inorganic carbon pools, and (2) prescribe overlay %CO_2 and flow to achieve a specified gas-phase pCO_2 (e.g., the 100 mmHg measured at large scale), even when the sparge remains air.

Assumptions

A) Core chemistry

- Fixed temperature = 37 °C: Constants are set for 37 °C (pK'a ≈ 6.10; Henry's constant $H_{37} \approx 0.0309$ mM/mmHg). Update them if temperature changes.
- Henderson–Hasselbalch applies to CO_2/HCO_3^-: pH is governed by carbonic acid vs. bicarbonate/carbonate. Accuracy drops with very strong non-carbonate buffers or rapid transients.

- Base equivalents only from bicarbonate/carbonate: We use B_eq = $[HCO_3^-]$ + $2[CO_3^{2-}]$. Other buffers (phosphate, amines) are ignored unless you extend the model.

- Lactate is an acid load: Lactate (g/L) is converted to mM on the anion basis (MW 89.07). If your assay reports lactic acid, use MW 90.08.

B) Linking liquid and gas CO_2

- Henry's law is linear at 37 °C: $dCO_2 = H_{37} \times pCO_2$. Media composition/foaming can shift effective solubility.

- Headspace pressure ≈ 1 atm unless specified: Gas fraction $y_{\text{base}} = \frac{p\text{CO}_{2,\text{base}}}{P_{\text{tot}}}$

use actual P_tot if reactor is pressurized or under vacuum.

C) Headspace overlay blending

- Steady state; CER cancels using baseline: We eliminate unknown CO_2 evolution by referencing the baseline, leading to

$$\boxed{X_{ol} = y^* + \frac{Q_{sp}}{Q_{ol}}(y^* - y_{\text{base}})}$$

- Well-mixed headspace: Overlay gas mixes before exhaust; poor mixing can cause off-gas lag vs. true headspace.

- Sparge is CO_2-free (air/air+O_2) in the basic model: If sparge contains CO_2, include it explicitly in the balance.

D) Scale-down fidelity

- Large-scale pCO_2^* is the target to reproduce: Use the measured plant headspace pCO_2 (e.g., 100 mmHg) as the benchmark; ensure probe placement/pressure are accurate.

- Mass transfer in SDM must be sufficient: If headspace pCO_2 matches but dCO_2 is low, increase overlay flow or %CO_2, or reduce total sweep (limit stripping).

E) Control & dosing simplifications

- Base top-up via bicarbonate/carbonate: Raising dCO_2 adds acid load; offset with $NaHCO_3$ (1 mEq/mM) or Na_2CO_3 (2 mEq/mM). Account for any strong-base pH control if present.

- Hold pH at the setpoint: All calculations assume the controller maintains the chosen pH (e.g., 7.10). Slow/saturated control will deviate.

2. Acid–Base Model (CO_2–Bicarbonate–Carbonate–Lactate)

We adapt the widely used semi-empirical formulation for bicarbonate-buffered media (37 °C). Let all concentrations be in **mM**.

2.1 Working balance

$$10^{(pH-pK_{a,CO2})}=\frac{B_{eq}-L}{dCO_2}\Rightarrow dCO_{2,req}=\frac{B_{eq}-L}{10^{(pH-pK_{a,CO2})}}$$

Where,

dCO_2=dissolved CO_2 in liquid (mM)
L=Lactate (mM)
B_{eq}=[HCO^{-}_{3}]+2[CO^{2-}_{3}] (mM);
$pK_{a,CO2}\approx$ 6.10 at 37 °C (tune ± 0.050-0.10 depends on cell culture medium).

Interpretation. For a fixed pH setpoint, rising lactate increases acid equivalents and therefore increases the required dissolved CO_2 to satisfy (1) unless $B_{eq}$is augmented by bicarbonate/carbonate addition.

2.2 Lactate unit conversion (when reported in g/L)

$$L[\mathrm{mM}]=\left\{\begin{array}{ll}1000\cdot\dfrac{\text{lactate (g/L)}}{89.07} & \text{(lactate anion)}\\ 1000\cdot\dfrac{\text{lactic acid (g/L)}}{90.08} & \text{(as acid)}\end{array}\right. \qquad (2)$$

3. Linking Dissolved CO_2 to Gas-Phase CO_2

At 37 °C:

$$d\mathrm{CO_2}\ [\mathrm{mM}]=H_{37}\left[\frac{\mathrm{mM}}{\mathrm{mmHg}}\right]\cdot p\mathrm{CO_2}\ [\mathrm{mmHg}];\ H_{37}\approx 0.0309 \qquad (3)$$

$$p\mathrm{CO_2}\ [\mathrm{mmHg}]\approx 7.6\times\%\mathrm{CO_2}\ (\mathrm{gas}) \qquad (4)$$

Combining (3) and (4):

$$d\mathrm{CO}_2\ [\mathrm{mM}] \approx 0.23484 \times \%\mathrm{CO}_2\ (\mathrm{gas}) \Rightarrow \%\mathrm{CO}_2 \approx \frac{d\mathrm{CO}_2}{0.23484} \qquad (5)$$

4. Headspace Overlay Blending to Match Large-Scale pCO$_2$

You observe: large scale $p\mathrm{CO}_2{}^{\backslash *} = 100\mathrm{mmHg}$; SDM baseline $p\mathrm{CO}_2, \mathrm{base} = 55\mathrm{mmHg}$. Let:

- Q_{sp}= sparge flow (air),
- Q_{ol}= overlay flow,
- X_{ol}= overlay CO$_2$ fraction to be set,
- y* is the target CO2 fraction based on large scale. $y^* = p\mathrm{CO}_2{}^*/760$
- y_{base} is simply the baseline mole fraction (or %) of CO$_2$ in the small scale.
  We get it by converting the baseline CO$_2$ partial pressure (in mmHg) to a fraction of total pressure:

$$\boxed{y_{\mathrm{base}} = \frac{p\mathrm{CO}_{2,\mathrm{base}}}{P_{\mathrm{tot}}}}$$

- If the reactor headspace is at ~1 atm absolute, $P_{\mathrm{tot}} \approx 760$ mmHg.
  That's why you saw $y_{\mathrm{base}} = p\mathrm{CO}_{2,\mathrm{base}}/760$.

Example: $p\mathrm{CO}_{2,\mathrm{base}} = 55$ mmHg$\Rightarrow$
$y_{\mathrm{base}} = 55/760 = 0.0724$= **7.24% CO$_2$** in the headspace.

Using a steady-state headspace material balance while eliminating unknown CER with the baseline condition yields a practical prescription:

$$\boxed{X_{ol} = y^* + \frac{Q_{sp}}{Q_{ol}}(y^* - y_{\mathrm{base}})} \qquad (6)$$

Feasibility: If $X_{ol} > 1$, increase Q_{ol}, or increase the oxygen proportion in sparger to reduce Q_{sp}.

5. SDM Implementation Workflow

Inputs

- pH setpoint; measured/forecast lactate; bicarbonate and carbonate (mM).
- Large-scale target pCO_2 (e.g., 100 mmHg).
- SDM baseline pCO_2 (without CO2 enrichment in the overlay) and gas flows Q_{sp}, Q_{ol}.

Step 1 — Acid–base requirement

Compute $B_{\text{eq}} = [\mathrm{HCO_3^-}] + 2[\mathrm{CO_3^{2-}}]$.
From (1), evaluate $d\mathrm{CO_2}, \mathrm{req}$for the chosen pH.

- If $B_{\text{eq}} - L \leq 0$, base is deficient; trim in bicarbonate/carbonate before pCO_2 matching.
- Otherwise, proceed.

Step 2 — Gas-phase target and expected dissolved CO_2

From the large-scale target $p\mathrm{CO_2}^*$, compute

$$d\mathrm{CO_2}^* = H_{37} \cdot p\mathrm{CO_2}^* \,; H_{37} \approx 0.0309 \text{ mmHg/ mM at } 37 \circ \mathrm{C}$$

Example (at $p\mathrm{CO}_2^* = 100$ mmHg):
$d\mathrm{CO}_2^* = 0.0309 \times 100 = \mathbf{3.09}$ mM.

Check that $d\mathrm{CO_2}^{\backslash *}$and $d\mathrm{CO_2}, \mathrm{req}$are **compatible** with your pH and buffers. If not, adjust B_{eq}(bicarbonate/carbonate) or consider a slightly different pCO_2 target with justification.

Step 3 — Overlay CO_2 setpoint

Measure/estimate the SDM **baseline** headspace CO_2 fraction

$$y_{\text{base}} = \frac{p\mathrm{CO}_{2,\text{base}}}{P_{\text{tot}}}.$$

With sparge flow Q_{sp}(air) and overlay flow Q_{ol}, set the required **overlay CO_2 fraction** $X_{ol}$by

$$\boxed{X_{ol} = y^{*} + \frac{Q_{sp}}{Q_{ol}}(y^{*} - y_{\text{base}})}$$

and convert to %CO_2 for the MFC: $\%\mathrm{CO}_2^{\text{overlay}} = 100\, X_{ol}$.

(If $X_{ol} > 1$, increase Q_{ol}, decrease Q_{sp}, or add CO_2 to the sparge.)

6. Illustrative Example

Given

- pH = 7.10
- Lactate = 1.8 g/L
- Bicarbonate = 29 mM;
- Carbonate = 0 mM
- $pK_a' = 6.10$
- H_{37} = 0.0309 mM/mmHg
- Large Scale Target pCO_2^* = 100 mmHg
- $SDM\ baseline\ pCO_2 = 55\ mmHg$
- $Q_{sp} = 0.002$vvm
- $Q_{ol} = 0.02$vvm

Step 1 — Check Acid–base requirement (unchanged)

$$R = 10^{(pH-pKa)} = 10^{(7.10-6.10)} = 10$$

$$d\mathrm{CO}_2, \mathrm{req} = \frac{B-L}{R} = (29-20.2)/10 = \mathbf{0.88\ m} \qquad\qquad\qquad \mathbf{M}$$

This just tells you: at pH 7.10, given B_{eq} and L, you'd "naturally" sit at **0.88 mM dCO_2**.Which is lower than the target of **3.09 mM. So Top of base is required.**

Step 2: Bicarbonate top-up Requirements ?

What is a base trim?

When you **raise dCO_2** (e.g., to match plant pCO_2 = 100 mmHg → **dCO_2*** ≈ 3.09 mM), you add carbonic-acid load. To keep **pH 7.10** at the same lactate and buffer, you must add **extra base equivalents** (usually $NaHCO_3$ or a little Na_2CO_3). That addition is the **base trim**.

How much base?

Use the bicarbonate balance (37 °C):

$$dCO_2, \mathrm{req} = \frac{B_{\text{eq}} - L}{10^{(\mathrm{pH}-\mathrm{p}K_a')}} \Rightarrow B_{\text{eq,needed}} = L + dCO_2{}^{*}\ 10^{(\mathrm{pH}-\mathrm{p}K_a')}$$

Where $B_{\text{eq}} = [\mathrm{HCO}_3^-] + 2[\mathrm{CO}_3^{2-}]$.

Given

pH = 7.10, $pK_a' = 6.10 \Rightarrow 10^{(\mathrm{pH}-pK_a')} = 10$
Lactate $L = 20.2\text{mM}$
Current $B_{\text{eq}} = 29\text{mM}$ (29 mM HCO_3^-, no CO_3^{2-})
Target $dCO_2{}^{*} = 3.09\text{mM}$

Compute

$$B_{\text{eq,needed}} = 20.2 + (3.09 \times 10) = 51.1\ \text{mM}$$
$$\Delta B_{\text{eq}} = B_{\text{eq,needed}} - B_{\text{eq,current}}$$
$$= 51.1 - 29 = 22.1\ mM$$

Convert to a practical addition

- **As $NaHCO_3$** (1 mEq base per 1 mM): add **22.1 mM $NaHCO_3$**
  → grams/L: 22.1 mmol/L × 84.01 mg/mmol $= 1.8 g/L$

- **As Na_2CO_3** (2 mEq base per 1 mM): so 22.1 mM/2 = **11.05 mM Na_2CO_3**
  → grams/L (anhydrous): $11.05 \times 105.99 = 1.17 g/L$

Step 3 — Overlay CO_2 setpoint

Large scale target: $p\mathrm{CO}_2^* = 100\mathrm{mmHg}$

$$\Rightarrow y^* = 100/760 = 0.1316$$

SDM baseline: $p\mathrm{CO}_{2,\mathrm{base}} = 55\mathrm{mmHg}$

$$\Rightarrow y_{\mathrm{base}} = 55/760 = 0.0724$$

SDM flows: $Q_{sp} = 0.002\mathrm{vvm}$, $Q_{ol} = 0.02\mathrm{vvm}$

$$\Rightarrow Q_{sp}/Q_{ol} = 0.1$$

Use

$$X_{ol} = y^* + \frac{Q_{sp}}{Q_{ol}}(y^* - y_{\mathrm{base}})$$

Compute:

- $y^* - y_{\mathrm{base}} = 0.1316 - 0.0724 = 0.0592$
- $\frac{Q_{sp}}{Q_{ol}} = 0.002/0.02 = 0.1$

So,

$$X_{ol} = 0.1316 + 0.1 \times 0.0592 = 0.1316 + 0.00592 = \mathbf{0.13752}$$

⇒**%CO_2 in overlay** $= 100 \times X_{ol} \approx \mathbf{13.75}\%$

(Feasible; well below 100%. If headspace/dCO_2 still lag due to stripping, raise Q_{ol}slightly or nudge X_{ol}up.).

Appendix A

Rationale for omitting $k_L a$ from the SDM pCO$_2$ model.

Our objective is a **setpoint** problem: reproduce the large-scale headspace $p\mathrm{CO}_2$ and maintain pH at the chosen setpoint. At steady state, the required quantities are governed by **gas composition and equilibrium** (overlay fraction/flows and Henry's law), not by transfer *rates*. Accordingly, we (i) prescribe the overlay %CO$_2$ to attain the target headspace fraction $y^* = p\mathrm{CO}_2^*/P_{\mathrm{tot}}$, and (ii) compute the **base equivalents** needed to hold pH given $d\mathrm{CO}_2$, lactate, and bicarbonate/carbonate.

We **do not explicitly include** $k_L a$ for two practical reasons:

1. **Headspace setpoint is independent of $k_L a$.** The steady-state headspace composition results from inlet gas fractions and overlay/sparge flows. $k_L a$ influences the *rate* at which the liquid approaches equilibrium, not the headspace composition we set.

2. **Bench-scale transfer is not rate-limiting under our conditions.** In <5 L reactors operated here, $d\mathrm{CO}_2$ closely tracks $H_{37}\, p\mathrm{CO}_2$ at steady state (rapid step responses; $d\mathrm{CO}_2^{\mathrm{meas}}/H_{37} p\mathrm{CO}_2 \approx 1$). Thus, **mass transfer is sufficiently fast** that CO$_2$ is not limiting for the steady-state setpoints we target.

As a safeguard, we **empirically verify** that measured $d\mathrm{CO}_2$ matches the equilibrium value implied by the headspace. If stripping or dynamics cause a systematic shortfall, we correct operationally—**increasing overlay %CO$_2$ or Q_{ol}**, or reducing total sweep—without introducing an uncertain $k_L a$ parameter. We would include $k_L a$ only for **transient predictions**, **co-sparged CO$_2$**, or scenarios where **steady-state $d\mathrm{CO}_2$ deviates materially from equilibrium**.