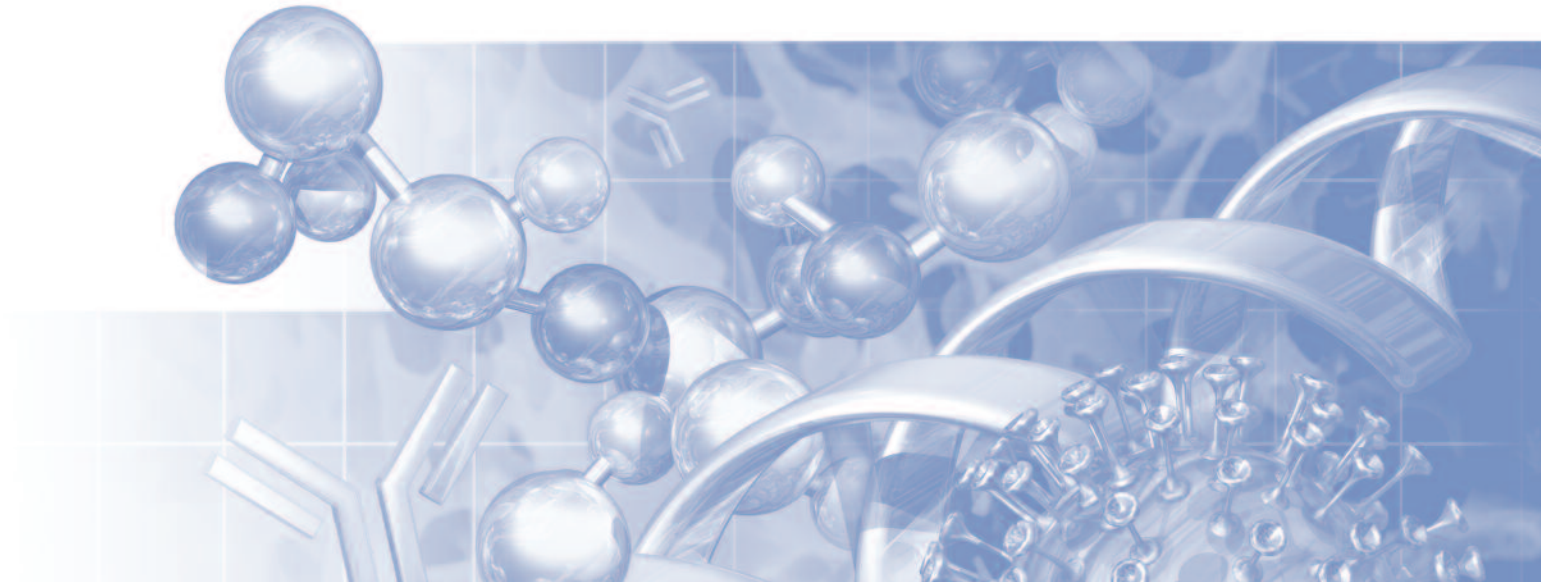
PALL Life Sciences

Application Note

USD 3006

Mass Transfer Coefficient (K_La) in the iCELLis® Nano Single-Use Bioreactors



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Introduction

In bioprocesses, the dissolved oxygen (DO) concentration is a key parameter to obtain optimal cell growth. In these processes, oxygen concentration is commonly controlled between 20 and 100%. This ability to regulate DO is dependent on the oxygen transfer rate (OTR) from the gaseous phase to the liquid phase, which is affected by the volume of the liquid inside the bioreactor (V), the difference in the oxygen concentration between the head space (C_{Sat}) and the liquid (C) and the volumetric mass transfer coefficient, K_La. This K_La gives direct information about the aeration capacity of a system.

$$OTR = K_La.\ V.\ (C_{sat} - C)$$

Each bioreactor system has its own specific K_La, and so has the iCELLis line bioreactors. The iCELLis platform, a scalable line of single-use high cell density bioreactors (Pall Life Sciences), is specially designed for adherent cell culture applications (e.g. recombinant protein and viral vaccine production) (Figure 1).

Central to the iCELLis bioreactor is the use of a compact fixed-bed pre-packed with medical grade proprietary microcarriers (polyester microfibers) providing a large growth surface area in a compact bioreactor volume. Surface area of each individual carrier is equivalent to 11.2 cm^2.

The iCELLis line of bioreactors is linearly scalable, from small scale (starting at 0.53 m^2) to large-scale manufacturing size (up to 500 m^2) (Table 1). As the bioreactor scale increases, the fixed-bed height remains constant while the diameter increases. Straightforward scale-up is then ensured by keeping the same culture parameters (pH, DO and t°), identical cell density (per volume and per surface area units) and similar cell culture conditions: compaction of carriers, linear speed through the fixed-bed and perfusion rate.

Table 1

Different configurations of the iCELLis nano bioreactor

iCELLis nano bioreactor features	Low compaction			High compaction		
Fixed bed volume (mL)	40	80	200	40	80	200
Surface area (m^2)	0.53	1.07	2.67	0.80	1.60	4.01
Volume of liquid inside the fixed-bed (mL)	37.1	74.5	186.1	35.6	71.3	178.5
Roller Bottle -850 cm^2 equivalent	6.2	12.6	31.5	9.3	18.7	47.2
Cell Stack 10 equivalent	0.8	1.7	4.2	1.3	2.5	6.3

The bioreactor is provided with a built-in magnetic drive impeller which enables the medium circulation through the fixed-bed from the bottom to the top, ensuring low shear stress and high cell viability. At the top of the fixed bed, the medium falls as a thin film down the outer wall where it takes up O_2 to maintain K_La in the bioreactor (Figure 1 and 2).

Figure 1

The semi-disposable iCELLis nano system (0.5 – 4 m^2)

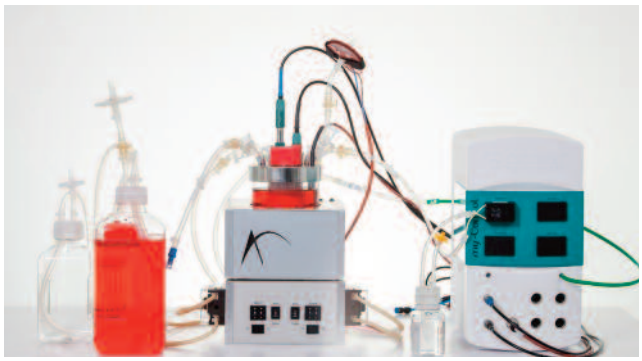


Figure 2

Liquid circulation pattern into the iCELLis nano bioreactor with a controller for DO, pH and T°

The mass transfer coefficient (K_La) of the iCELLis bioreactors is dependent on the height of the falling film (FF) which is directly linked to the working volume (Table 2). In the iCELLis nano bioreactor, this working volume is between 600 and 1,000 mL. In many applications, the iCELLis system is used under recirculation/perfusion mode which impedes a working volume of 860 mL in the iCELLis vessel (equivalent to 3 cm FF). This volume/height is controlled by the standard feed-out dip tube. Alternatively, the bioreactor can be filled to 1,000 mL which corresponds to a 1 cm FF. In the large scale iCELLis system, falling films of 2, 6 or 10 cm are applicable. In this application note, we describe K_La study in the iCELLis nano bioreactor for different working volumes.

Table 2

Falling film equivalences with the total liquid volume in the iCELLis nano bioreactor

Falling film height (cm)	Volume of liquid (mL)	Characteristic volumes
1	1,000	Maximum working volume
2	950	–
3	860	Recirculation loop volume
4	800	Inoculation volume
6	670	–

Method

All the experiments were performed on the iCELLis nano bioreactor with a 200 mL fixed-bed at compaction 1.5 using PBS solution at 37 °C and air flow set at 30 mL/min (usual air flow in the bioreactor). Prior to these tests, the DO probe was calibrated in two points (0% and 100 % air saturation). For each experiment, dissolved oxygen (DO) was depleted from the PBS by N_2 gas injection in the headspace of the bioreactor, with stirring activated, while the DO was monitored. Once the DO was at 0%, the N_2 was quickly purged from the bioreactor headspace by air addition at 30 mL/min. Increase in the measurement of DO was monitored by the iCELLis controller and used to determine K_La using the Dynamic Response Method.

Results / Discussion

Important factors affecting the K_La of the iCELLis nano are the height of the falling film (FF) coupled with the bioreactor working volume (the lower the volume, the higher the FF) and the liquid flow rate through the fixed-bed (for more details, see the related application note, Characterization of the liquid speeds in the iCELLis nano bioreactors – reference USD 2942). In this study, K_La was determined using falling film heights of 3 and 6 cm which correspond to the working volumes of 860 and 670 mL, respectively, at various liquid flow rates with PBS. K_La results based on these tests are summarized in the Table 3.

Table 3

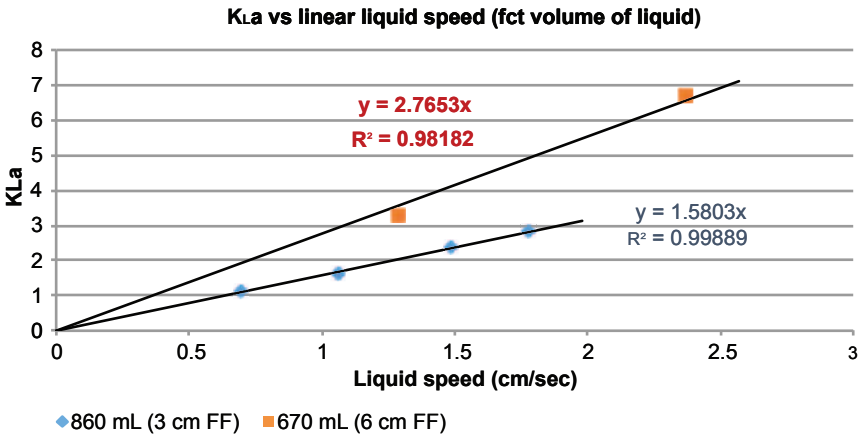
Measured K_La for 3 and 6 cm falling films for specific liquid speeds in the iCELLis nano system (200 mL C 1.5)

860 mL (3 cm FF)		670 mL (6 cm FF)	
Flow rate (cm/sec)	K_La	Flow rate (cm/sec)	K_La
0.70	1.13	1.29	3.28
1.06	1.64	2.37	6.71
1.49	2.36	-	-
1.77	2.82	-	-

As shown in Figure 3, the oxygen transfer rate is proportional to the FF height. This graph could be used as a guide to select the specific liquid flow rate required to allow sufficient oxygen transfer to the cells. Table 4 shows estimations of K_La requirements for specific cell lines in the iCELLis nano bioreactor (200 mL fixed-bed at compaction 1.5 (4 m^2)) with 3 and 6 cm FF and the corresponding liquid flow rates required. Estimations for the other iCELLis sizes can be performed identically.

Figure 3

Correlation between the K_La and the liquid flow rates (function of the liquid volume) in the iCELLis nano bioreactor (200 mL C 1.5)



Aside from the parameters discussed above, it is also important to consider the impact of cell growth on the liquid flow rate, since increased cell density will hinder flow through the fixed-bed and will directly impact the K_La. Preliminary data suggests that the liquid flow rate could decrease by 20% or more between the seeding time and maximum cell density reached (data not shown). Other factors which could affect K_La include temperature, medium composition, viscosity, turbulence and the presence of foam. In order to determine the K_La in a specific cell culture medium, the experiments described above could be repeated using specific culture media instead of PBS. To avoid the results being affected by microbial growth, use of antibiotics is recommended.

Table 4

Theoretical K_La requirements for specific cell lines and its liquid speeds equivalences in the iCELLis nano bioreactor 200 mL C 1.5

Cell line (OUR – mmol/1e9vc/h)	3 cm Falling Film (860 mL)		6 cm Falling Film (670 mL)	
	Theoretical K_La required (h^{-1})*	Theoretical liquid flow rate required (cm/sec)	Theoretical K_La required (h^{-1})*	Theoretical liquid flow rate required (cm/sec)
CHO (0.15)	2.92	1.85	3.75	1.36
BHK21 (0.20)	3.89	2.46	5.00	1.81
VERO (0.24)	4.67	2.95	6.00	2.17

**The theoretical K_La required are calculated for a cell density of $3x10^5$ cells/cm^2 and with air flow at 30 mL/min, DO set-point at 50%*

Tips / Formula

How do I calculate the theoretical K_La required for my cells?

Determine the specific Oxygen Consumption Rate (cOUR) of your cells (expressed in $mmol.h^{-1}.10^{-9}$ viable cells)	0.15 $mol.h^{-1}.10^{-9}$ viable CHO (this parameter is constant for a same cell line)
Determine the oxygen solubility at your working temperature (mmol/L)	0.19 mmol/L at 37 °C
Determine the maximum pO_2 in your headspace (for iCELLis nano bioreactor this parameter is constant) (% O_2)	30 mL/min of Air + 200 mL/min of O_2 = 89.6% O_2
Define your DO set-point (%)	50%
Set the maximum cell concentration achieved in the bioreactor ($cells/cm^2$)	300,000 $cells/cm^2$
Identify the working parameters of your iCELLis bioreactor	iCELLis nano bioreactor 4 m^2 with 860 mL liquid
Calculate the quantity of O_2 required per hour (mmol/h)	= (300,000 $cells/cm^2$*40,000 cm^2)x10^{-9}x0.15 = 1.8 mmol/h
Calculate your K_La as follows:	$KLa = \frac{\text{quantity of } O_2/\text{hour}}{\{\text{volume of IC nano } (\frac{(\text{Max } pO_2 - (O_2 \text{ solubility x DO set} - \text{point}))}{100}\}}$

How to determine the stirring speed required for achieving the theoretical K_La?

Let us consider the case of VERO cells grown at a maximum cell concentration of 300,000 $cells/cm^2$ in any type of iCELLis nano bioreactor with 860 mL working volume. Here, the theoretical K_La required is 4.67 h^{-1}. This value is given for DO set-point at 50% with a gas flow of 30 mL/min Air and 200 mL/min O_2. To get this K_La in the bioreactor, use the relationship between the K_La and the liquid flow rate through the fixed-bed (Figure 3).

For 860 mL working volume: K_La (h^{-1}) = 1.5803x liquid speed (cm/sec)

Knowing the liquid flow rate as well as the bioreactor type (fixed-bed height, compaction rate, working volume), the liquid flow rate can be easily converted in stirring speed (RPM) using the dedicated application note (Characterization of the liquid speeds in the iCELLis nano bioreactors – reference USD 2942).

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