- Sample -

Mixing Technology Development Program

15,000 liter Bioreactor Gas Dispersion, Oxygen Transfer and Blending Studies

Lightnin

Rochester, New York

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November 28, 2001
Rev1: 12/13/01

Program Objective

Evaluate the full-scale gas dispersion, mass transfer and blending performance of the proposed agitator design for the 15,000 liter bioreactors to optimize impeller type and placement, and thus insure desired performance. In particular, the use of a single impeller will be validated, with emphasis on use of a high efficiency, low shear hydrofoil to minimize possible cell damage.

Program Outline

- Prepare computational fluid mixing (CFM) models for desktop evaluation of flow patterns and energy dissipation for various impeller options
- Qualitative evaluation of blending and gas dispersion performance in a small-scale tank (17.5" diameter; 80 liter working volume)
- Qualitative and quantitative evaluation of gas dispersion, mass transfer, and blending in a near full-scale tank (96" diameter; 12,000 liter working volume)
- Prepare test documentation: videotape, data summary, report, etc.
- Meet with client to review findings and finalize agitator design

Proposed Test Program

1. Prepare CFM models to benchmark the proposed full-scale agitator configuration, and to evaluate flow patterns and energy dissipation over a range of operating speed and impeller configurations.

   The following variables will be examined using CFM and subsequent small and full-scale models; note that all models will use top-entry agitators, since the software and physical models are not configured for bottom-entry drives.

   - Impeller number: single A315 (A320) and A310 impellers at two different locations; dual A315 (A320) impellers at preferred locations.
   - Operating speed: 18 and 32 rpm (corresponds to the use of 42" diameter impellers and the specified tip speed range of 200-350 ft/s); 56 rpm (maximum (60 Hz) speed for the proposed drive)
   - Gas rate (not applicable for the CFM model): 120-240-450 slpm

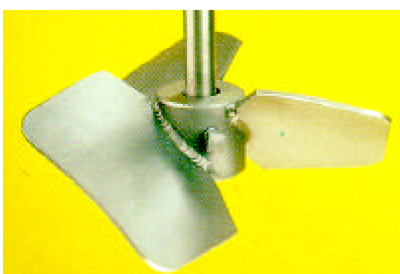
2. Qualitative evaluation of gas dispersion and blending performance in a small-scale acrylic tank: Operate geometrically scaled impellers (A315's (A320's) and A310's) at appropriately scaled-down operating speeds and gas rates to document gas dispersion and blending. F68 surfactant will be added at the appropriate concentration to properly simulate bubble generation and gas dispersion. Alternate sparge geometries as proposed and agreed upon will be evaluated. Baffle number (two versus four) will be evaluated to minimize tank internals while insuring proper top to bottom mixing. Blending performance will be evaluated using a dye indicator and acid-base addition.

3. Qualitative and quantitative evaluation of gas dispersion, mass transfer and blending performance in a near full-scale steel tank: The tank will be equipped with impellers of the appropriate size; the impeller type (3-bladed A320 or 3-bladed A310) will be a function of pre-test results from the small-scale tests. F68 surfactant will be added at the appropriate concentration to properly simulate bubble generation and gas dispersion. Sparge and baffle geometry as optimized in the small scale tests will be installed.

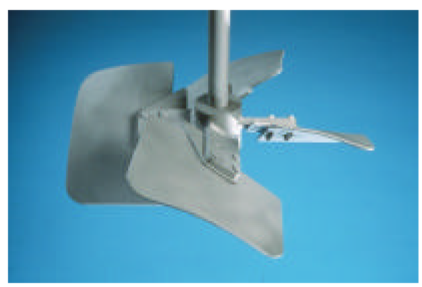
    - Impeller speed and torque (thereby yielding delivered power) will be monitored using available instrumentation
    - Visual evaluation of gas dispersion
    - Determination of oxygen mass transfer coefficient (K_La) using a non-steady state technique (ASCE Standard for the Measurement of Oxygen Transfer in Clean Water)
    - Evaluation of blending performance via addition of a salt tracer and in situ conductivity probes
    - Determination of local velocity at selected locations using either calibrated propeller or magnetic velocity probes.

4. Experiments will be documented on videotape, and a summary report will be prepared to present data, findings and recommendations.

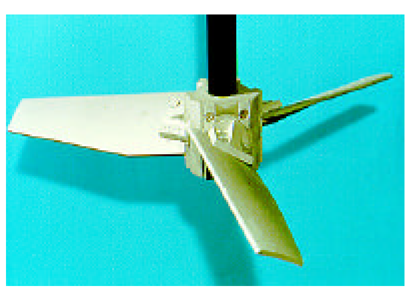
5. A follow-up review meeting will be scheduled as appropriate to discuss findings and recommendations, and to refine the agitator design as warranted.



Lightnin A320



Lightnin A315



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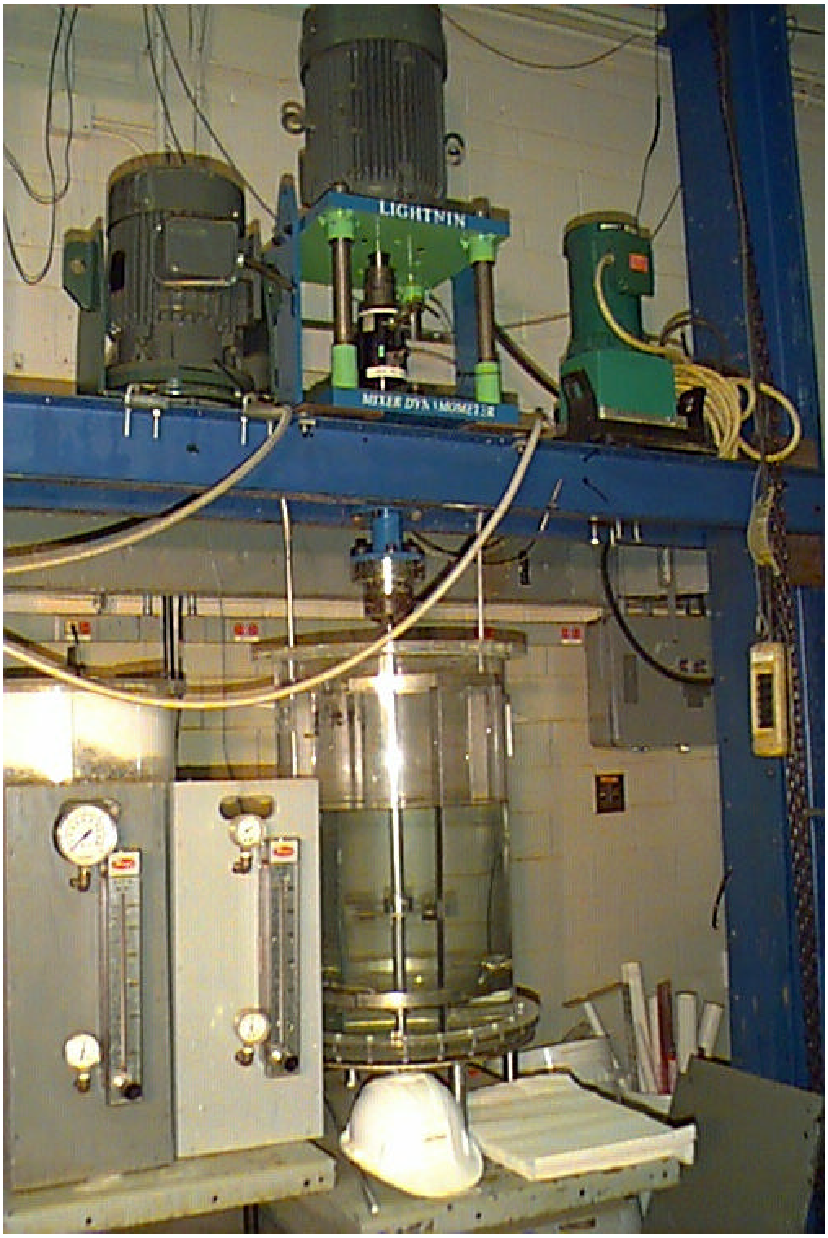
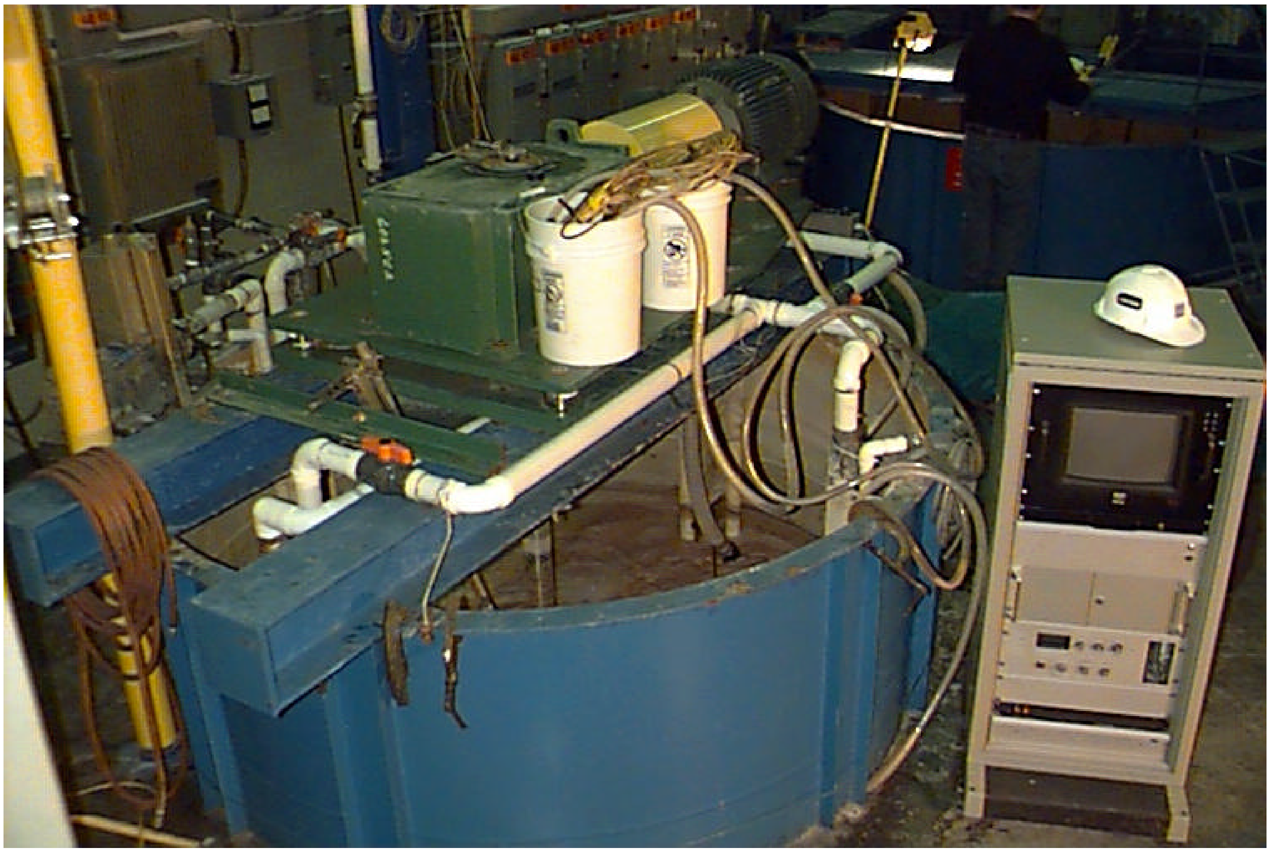


18" Test Tank

with

Dynamometer, Air Sparging and Removable Baffles



8’ Test Tank

with

Torque & Speed Measurement,
Mass Transfer & Blend Time Instrumentation,
Gas Sparging & Removable Baffles

Report
January 22, 2002

CONFIDENTIAL INFORMATION

For: ___________________

Cell Culture Bioreactor Process Study

LIGHTNIN Order # ___________

By,

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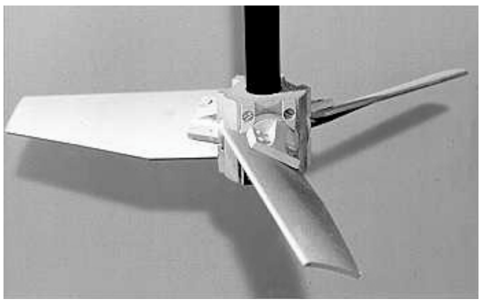
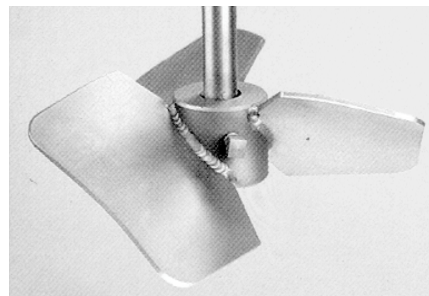
Introduction

The objective of the test program was to demonstrate the performance characteristics of LIGHTNIN A310 and A320 impellers in a shear sensitive cell culture application. Due to the relative low mixer power level and sparged air rate LIGHTNIN's 96" diameter test tank was used due its close scale reference to the client's planned ___" diameter vessel. Initial small scale tests were performed in LIGHTNIN's Process Technology Laboratory in 17-½" diameter Plexiglas vessels to visually evaluate performance differences between single and dual impeller configurations. Due to time constraints testing in the 96" diameter vessel was limited to dual impeller configurations using A310 and A320 impellers.



LIGHTNIN A310



LIGHTNIN A320

Both impeller systems were evaluated on relative blending and mass transfer performance as well as overall visual evaluation of the gas and liquid flow pattern in the 96" diameter vessel. This report covers work performed in the 96" tank only, additional work was completed in the smaller 17-½" that is not reported here.

Scale Down

The full scale bioreactor is currently designed at ___” diameter and thus not much larger than the 96” test scale. This greatly simplifies scale down criteria. The primary scale parameter is impeller tip speed which has been set at 350 fpm maximum with 200 fpm as the lower limit. The secondary process parameter is air flow rate through the sparge, it is scaled on VVM. While there is concern that air flow rate scale down on a VVM basis will lead to reduced k_La values at smaller scale this is not considered a significant contribution here since the scales are very similar. The following table details the scale parameters for the geometric similarity scale down of the vessel and associated process scale parameters:

PARAMETER	15,000 LITER	LIGHTNIN 96” TANK
Tank Diameter (T)	___”	96”
Liquid Height (Z)	___”	110”
Process Volume (V)	15,000 liter	12,320 liter
Z/T	___	1.15
Impeller Diameter (D)	__” A315 Quoted	40” A320, 42” A310
D/T	0.50	0.42 & 0.44
Speed [RPM]	56 Max, 30 Min Quoted	33 & 32 Max, 20 & 18 Min
Tip Speed [FPM]	750 Max, 400 Min	350 Max, 200 Min
Air Sparge Rate [lpm]		480 Max, 140 Min
VVM		0.039 Max, 0.011 Min

Because the volume ratio between the full scale and laboratory scale equipment is 1.22 the experimental data from the 96” tank requires very little scale up compromise to predict full scale operational data. The smaller impeller diameter was agreed to prior to test work commencement. Impeller, sparge diameter and location, and DO and conductivity probe locations are summarized as follows. Refer to Appendix A for additional detail.

PARAMETER	LIGHTNIN 96” TANK
Impeller Off Bottom, A310 & A320	23”
Impeller Spacing, A310 & A320	30”
Sparge Pipe Design	¾” Diameter Pipe, 33.3” long centered under shaft, (22) 1/16” holes
Sparge Ring Design	¾” Diameter Pipe, 33.3” diameter centered, (22) 1/16” holes
Baffles	Quantity 2,
DO Probe Location	8” Off Wall, 60” Off Bottom
Conductivity #1 Location	28” Off Wall, 15” Off Bottom
Conductivity #2 Location	28” Off Wall, 60” Off Bottom
Conductivity #3 Location	28” Off Wall, 100” Off Bottom
Conductivity #4 Location	2” Off Wall, 36” Off Bottom
Conductivity #5 Location	5” Off Wall, 73” Off Bottom

The selection of impeller tip speed as one of the main scale criteria was a customer requirement due to potential for cell damage. Discussions were held regarding the validity of tip speed as the single predictor for maximum and average shear. From these discussions it was concluded that for the purposes of the experiment tip speed would be held constant in order to baseline the test. Later evaluation of the shear performance of the A310 and/or A320 impeller in process may allow for higher tip speeds and thus mass transfer and blending performance. Small scale work indicated that the mixers as described above are capable of providing excess k_La to the process at time and further discussions is tabled at this time.

Conclusions & Recommendations

The following basic criteria were set by the client:

1. Tip speed not to exceed ___ fpm
2. Mass transfer coefficient in water with xxx surfactant identical to process k_La. Process design k_La to be __ hr^{-1}.
3. Air feed rate not to exceed ___ lpm full scale.
4. Blend time not critical since process has slow kinetics and respiration rate. Actual value is not defined but will be evaluated upon data generation from conductivity data.

Based on the above criteria the data generated from experiments in the 96" vessel at LIGHTNIN's facility in Rochester, NY allows the following conclusions:

1. A tip speed of ___ fpm with either dual __" A310 or __" A320 impellers provides for complete vessel motion, gassed or ungassed.
2. The mass transfer requirement can be met with any tested impeller configuration and speed, and at air flow rate above ___ lpm. Note that the ___ RPM case with dual A310's and ___ lpm air resulted in a measured k_La of _._ hr^{-1}. Based on the testing methodology using sulfite oxidation to measure k_La and the fact that this point drops from the developed correlation for k_La there is some concern that this configuration meets the minimum process mass transfer requirement.
3. The maximum blend time measured was ___ seconds for the __ RPM, ___ lpm dual A310 case. Based on statements from the client, this meets the process requirement. Note that a 3-minute blend time is reasonable based on three volume turnovers to achieve a blended tank (3,254 gallon tank volume, 3,200 gpm primary impeller pumping capacity).
4. Visual observation of the vessel flow pattern for the __ RPM dual A310 selection with either ___ lpm or ___ lpm air showed a relatively weak flow pattern and relatively unhindered gas rise to the surface. Depending on the process requirement at this low mixer speed the A310 may not be an adequate process selection.
5. Based strictly on client stipulations 1-4 above it appears that either the A310 or A320 selections as tested are adequate for the process.

Blending

The table in Appendix B describes the basic process conditions tested for various data runs. Conductivity data exists for the labeled runs. Note that the data represents normalized conductivity data to allow for data interpretation. As the procedure involved the surface addition of undissolved sodium sulfite salt the blending data should be used more as a relative measure of blending performance than absolute blend times. Nonetheless, as discussed above the measured blend times are consistent with known correlations for blend time calculation in low viscosity applications.

The data was generated at a frequency of 50 Hz and collected for over 5 minutes for each run. The attached data plots show the first 200 seconds or 10,000 data points each. Normalization procedure was to set the final steady state conductivity of each probe at 100%, all other points are relative to this value. The raw data spread was on the order of 5% variation for probes 1-4, probe 5 exhibited higher measurement gain and thus produced data reading up to 100% higher conductivity of the non normalized data.

Mass Transfer

The mass transfer coefficient, k_La, was measured using the method described by Gigas & Post in their 1998 paper "Application of Batch Method k_La Determination in Mechanically Agitated Vessels" presented as paper 188f at the 1998 AIChE Annual Meeting in Miami.

All runs proved to be repeatable with several control runs to determine data validity. A review of the attached data shows that all runs, independent of impeller selection can be correlated to within ±15% accuracy using the following equation:

$$k_L a = K \cdot \left(P/V\right)^a \cdot SGV^b$$

$$k_L a \quad \left[hr^{-1}\right]$$

$$P/V \quad \left[HP/1000\,gal\right]$$

$$SGV \quad \left[ft/\min\right]$$

K, a, b: Experimentally-determined constants for this test

This result is consistent with data from other experiments in air-water systems at this scale. The direct translation to the client's bioreactor system is difficult without oxygen solubility and k_L data. Indications from several runs are that mass transfer is reduced by approximately 30-40% with the addition of 1x surfactant based on our measuring methodology. Careful analysis of the DO profile during the runs with surfactant points to an interaction between the sulfite, surfactant, and DO probe and clear conclusions cannot be drawn with characterization of the test procedure with this particular surfactant.

Note that the low gas rate, low power A310 case (Set #2, Point 7) produces a k_La much lower than predicted by the correlation. This data point coupled with the visual observation of the gas distribution during this run indicates that the mixer was flooded at this point. It is the writer's opinion that this data point represents the region where the mixer is no longer effectively managing the process.

Computational Fluid Mixing

M98000-0001A A315/A315-28 D1= 1067 mm

15k liter Bioreactor

Drive: 14 Q 0.6

Impeller speed

= 32 RPM CCW

Powe·/Volume

= 0.1233 hp/kgal

= 0.0243 kW/m^3

Shaft Power

= 0.489hp

= 0.365Kw

Turnovers/min

= 3.775

Reynolds Number

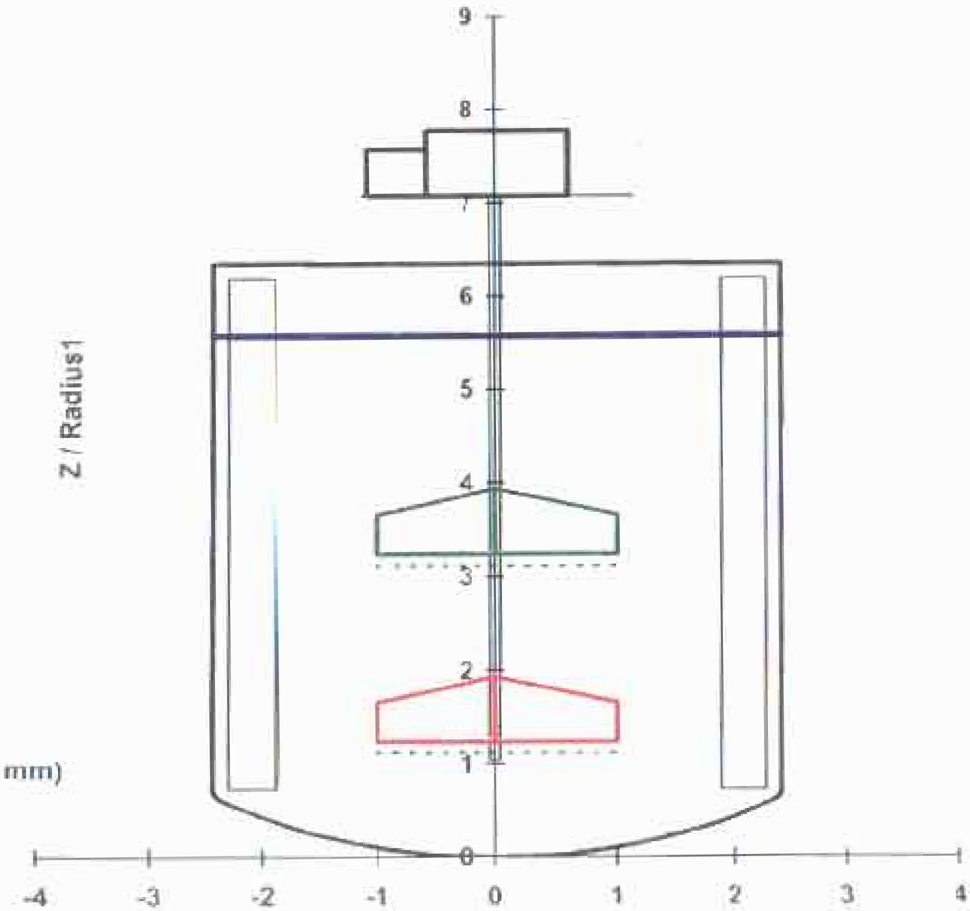
Main Impeller #1

= 333873

Tank Diameter: T

= 102 inch (2591 mm)

pmk



DM/T= 0.412

Mounting Surface Elevation:

= 148.6 inch (3774 mm) Elev.

Liquid Elevation: H

= 117 inch (2972 mm) Elev.

A315-28

D2= 42 inch (1067 mm)

C2= 74 inch (1880 mm) Elev

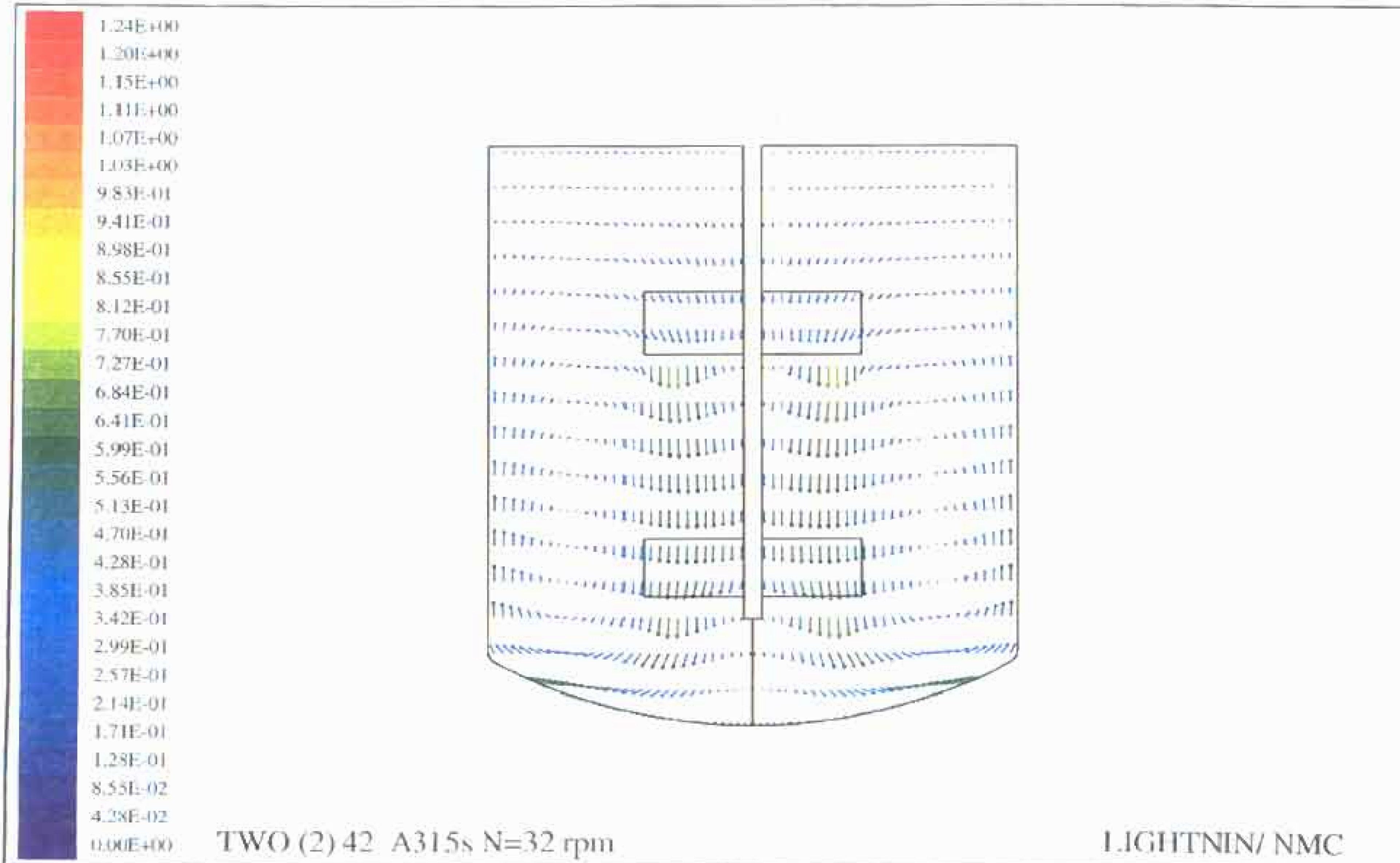
A315-28

D1= 42 inch (1067 mm)

C1= 32 inch (813 mm) Elev.

Zero Elevation at Inside of Tank Bottom

Radius / RadiusM



M2001000-0264A A315/A315-28 D1= 1067 MM

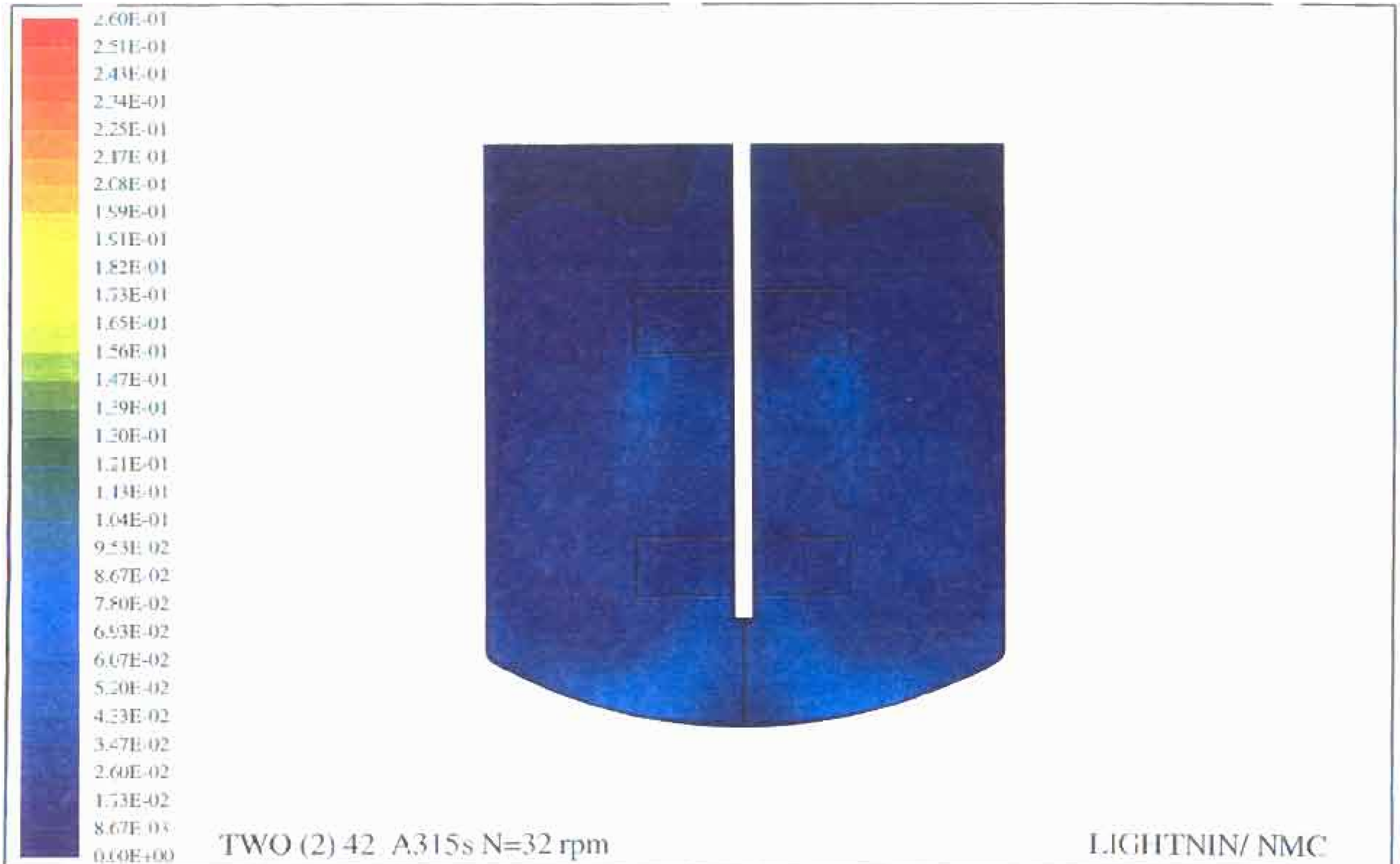
Velocity Vectors (M/S)

Max = 1.240E+00 Min = 0.000E+00

Dec 03 1901

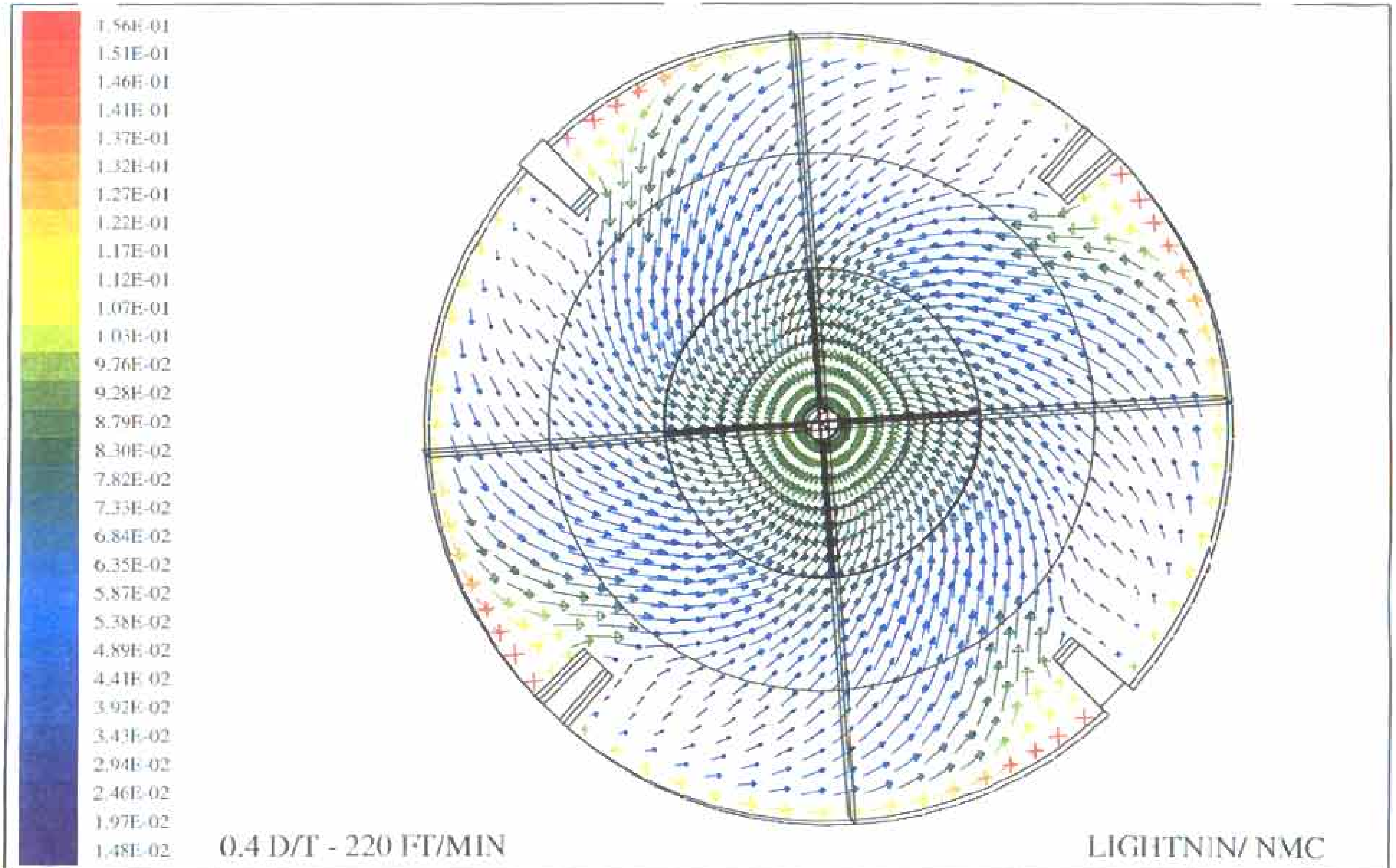
Fluent 4.56

Fluent Inc.

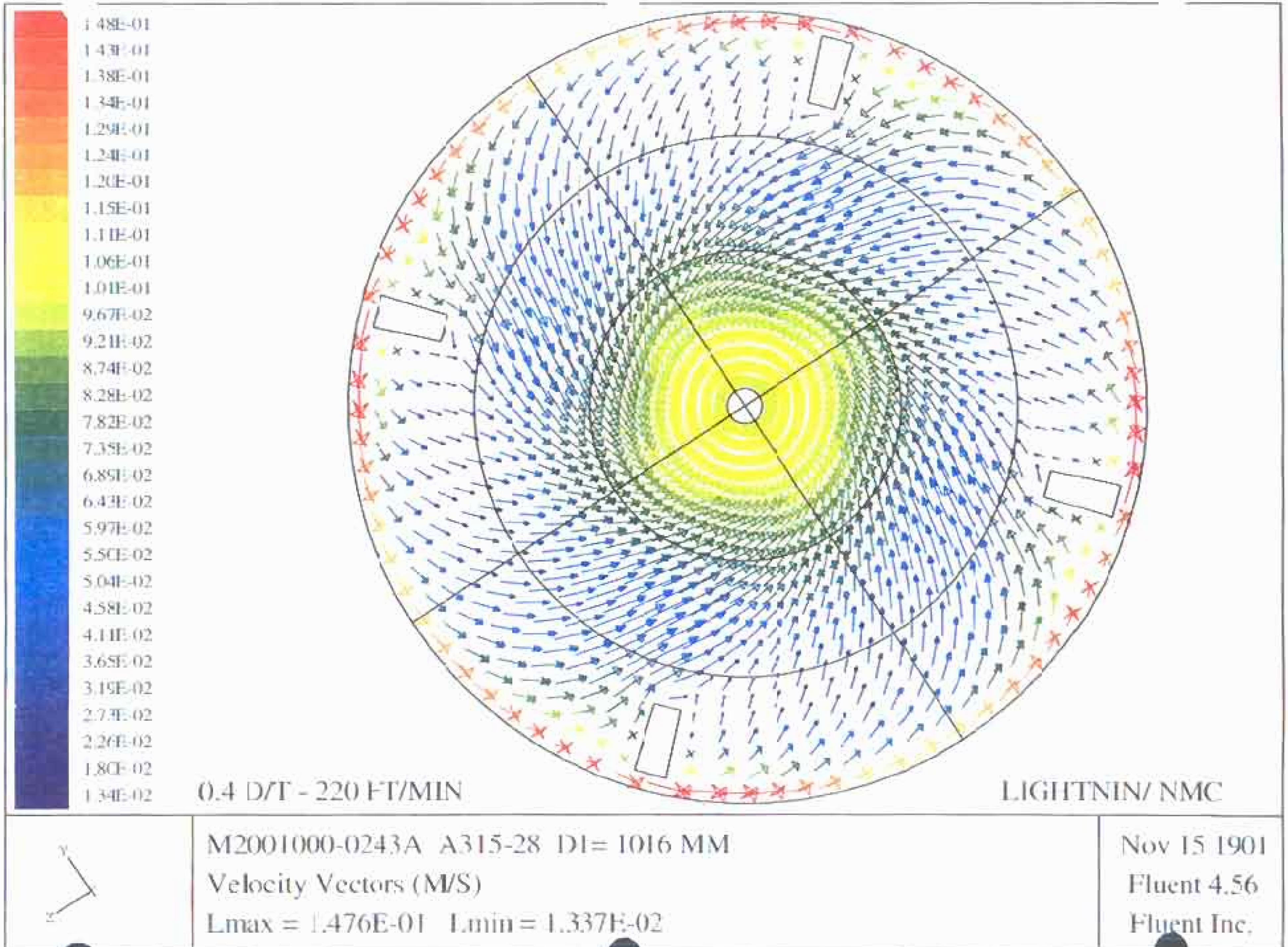


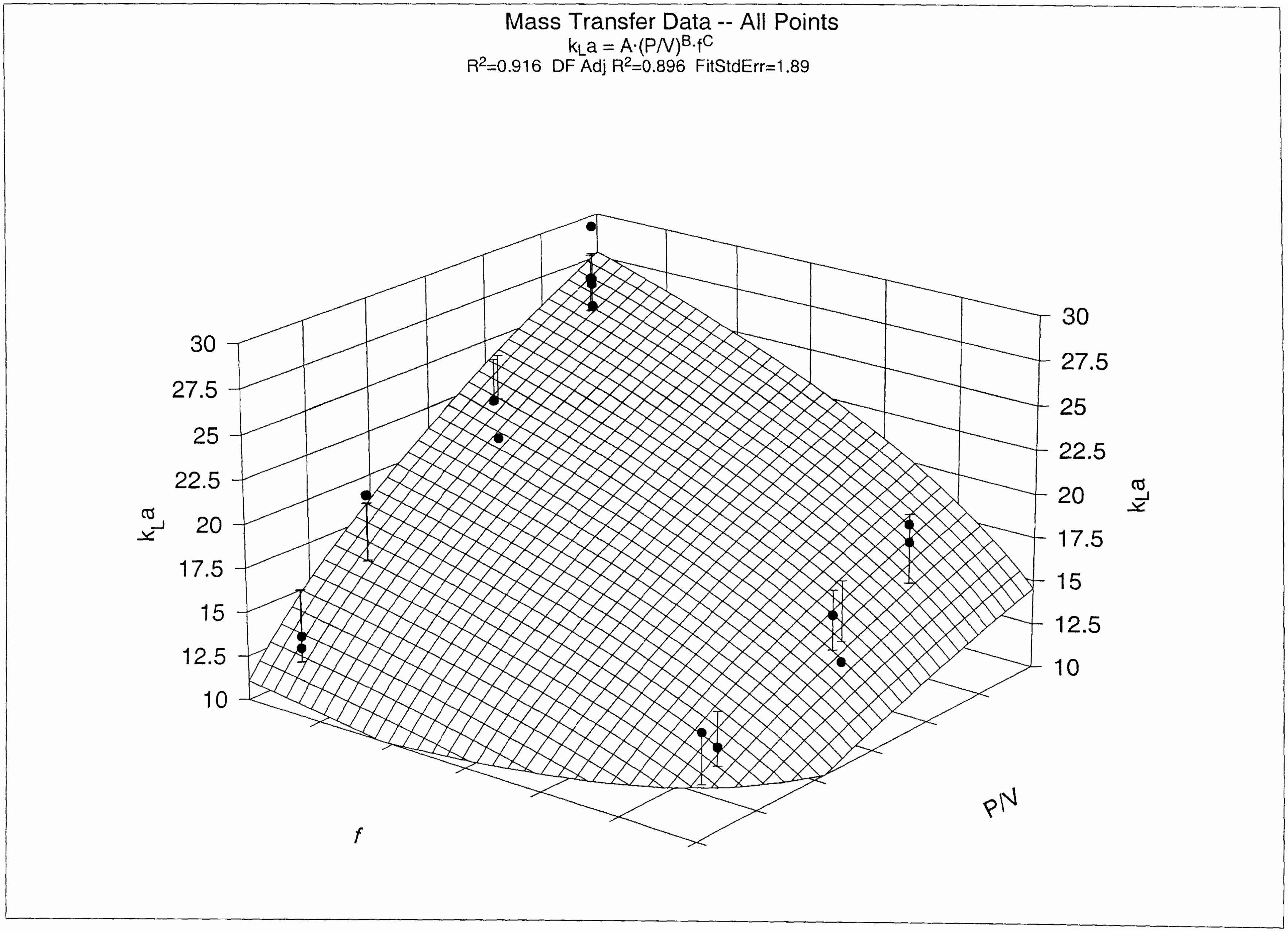
M2001000-0264A A315/A315-28 D1= 1067 MM
K.E. Of Turbulence (M2/S2)
Max = 2.600E-01 Min = 0.000E+00

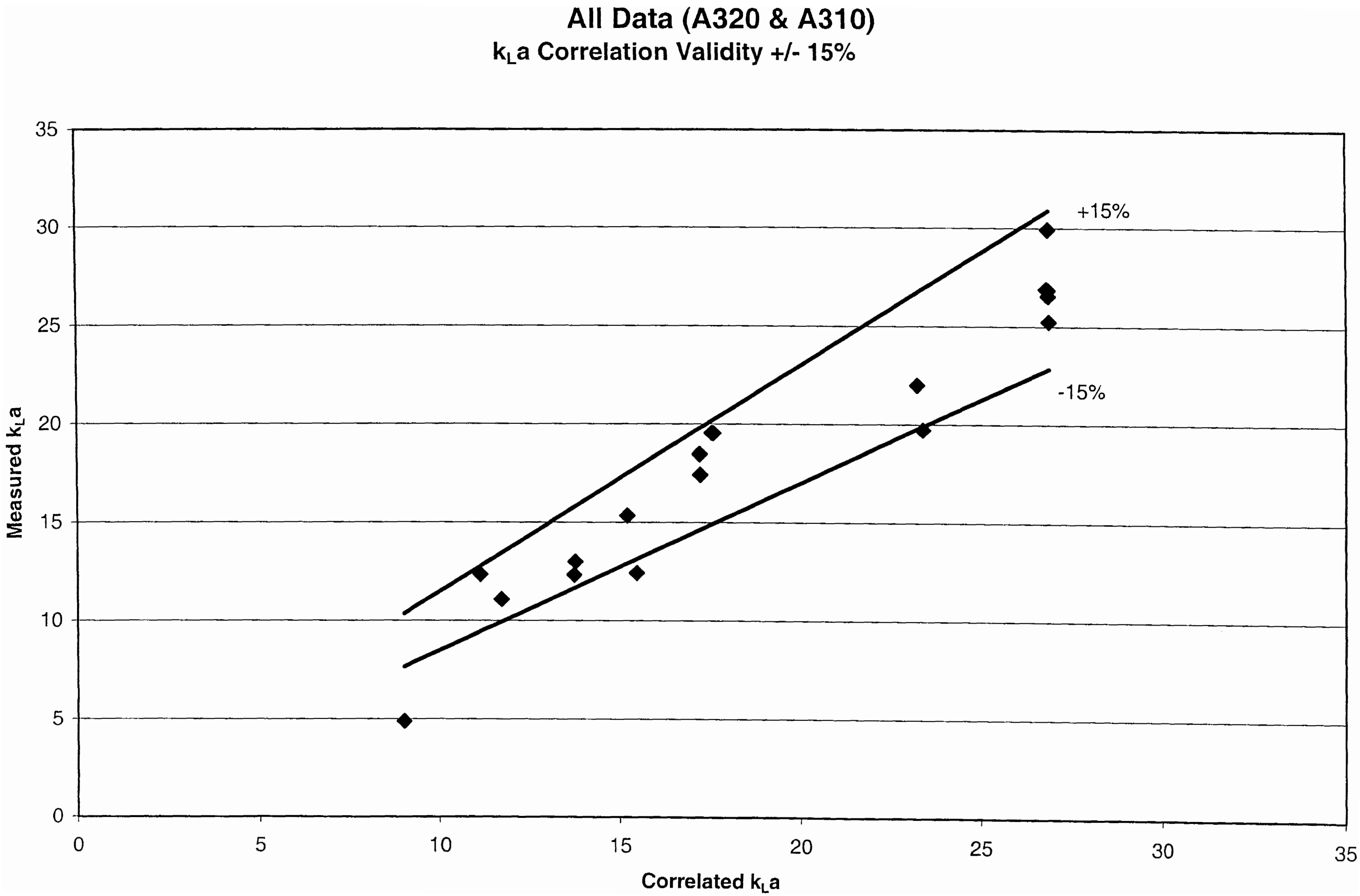
Dec 04 1901
Fluent 4.56
Fluent Inc.

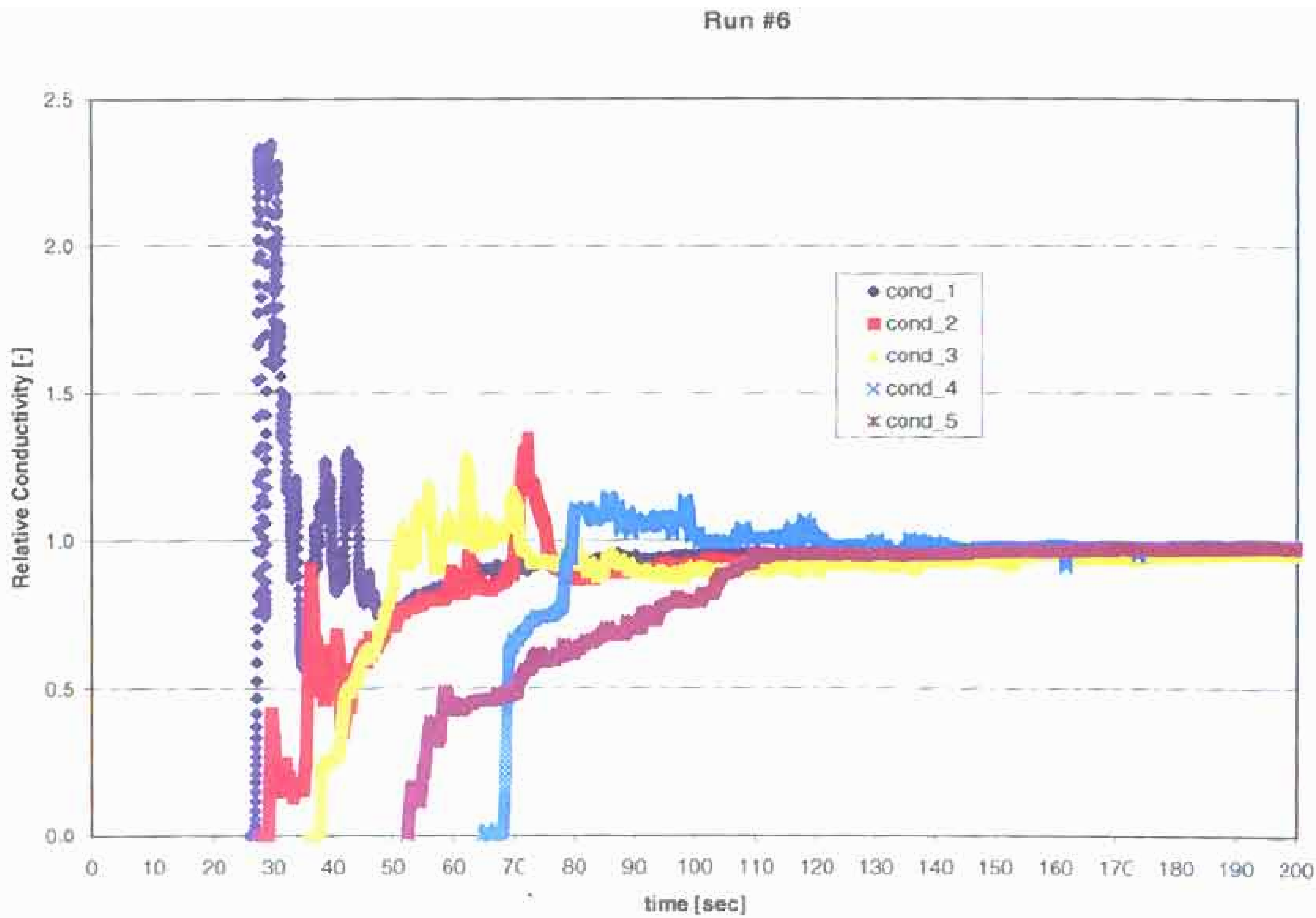


	M2001000-0246A A315-28 DI= 1016 MM Velocity Vectors (M/S) Lmax = 1.561E-01 Lmin = 1.482E-02	Nov 16 1901 Fluent 4.56 Fluent Inc.


1.48E-01
1.43E-01
1.38E-01
1.34E-01
1.29E-01
1.24E-01
1.20E-01
1.15E-01
1.11E-01
1.06E-01
1.01E-01
9.67E-02
9.21E-02
8.74E-02
8.28E-02
7.82E-02
7.35E-02
6.89E-02
6.43E-02
5.97E-02
5.50E-02
5.04E-02
4.58E-02
4.11E-02
3.65E-02
3.19E-02
2.73E-02
2.26E-02
1.80E-02
1.34E-02
0.4 D/T - 220 FT/MIN
LIGHTNIN/ NMC
M2001000-0243A A315-28 DI= 1016 MM
Velocity Vectors (M/S)
Lmax = 1.476E-01 Lmin = 1.337E-02
Nov 15 1901
Fluent 4.56
Fluent Inc.


Mass Transfer Data -- All Points
k_La = A·(P/V)^B·f^C
R²=0.916 DF Adj R²=0.896 FitStdErr=1.89
30
27.5
25
22.5
20
17.5
15
12.5
10
k_La
f
P/V


All Data (A320 & A310)
k_La Correlation Validity +/- 15%
+15%
-15%
Measured k_La
Correlated k_La
0
5
10
15
20
25
30
35



Run #6
2.5
2.0
1.5
1.0
0.5
0.0
Relative Conductivity [-]
cond_1
cond_2
cond_3
cond_4
cond_5
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
time [sec]