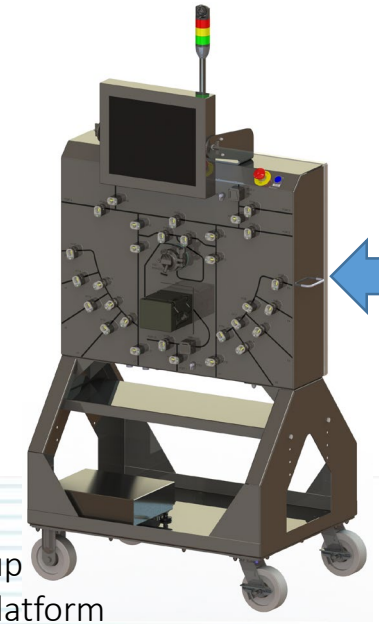
Iodixanol Gradient Scale Up Using Large Scale Ultracentrifugation

Sandra Meriño

Director of Global Strategic Solutions

Alfa Wassermann Promatix 1000™ Bio-purification system

Alfa Wassermann AFH-KII automated ultracentrifuge is a scalable centrifuge that is used in cGMP manufacture of gene therapy products



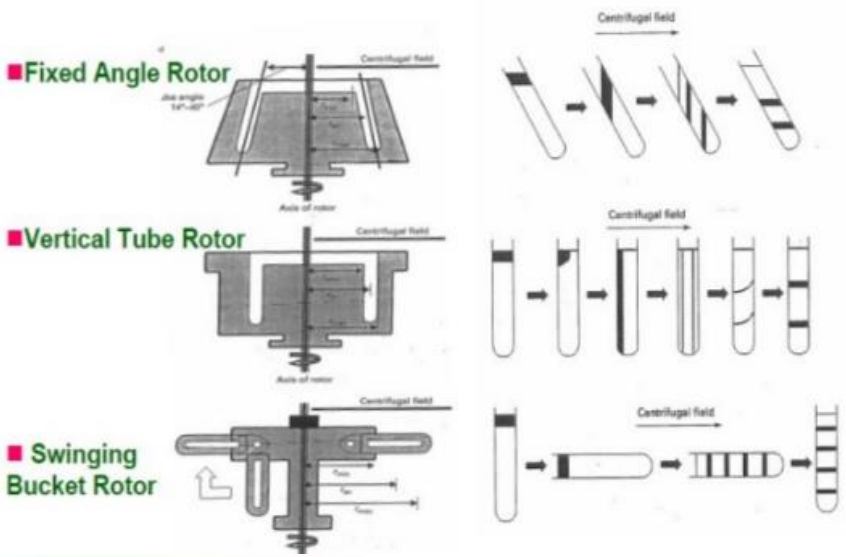
AWST Group
Software platform

Air drive KII

Laboratory scale ultracentrifuges allow a vast range of purifications to be made at a small scale – but these are not scalable for manufacturing

These rotors are limited by their capacity; too many runs would be needed to make a manufacturing batch of product at sufficient volume

Principle: Fill tube – spin tube – collect from tube



		Type of separation		
Type of Rotor	Pelleting	Rate-zonal sedimentation	Rate-zonal flotation	Isopycnic
Fixed-angle	Excellent	Limited	Good	Variable*
Near vertical	NS	Poor	Good	Variable*
Vertical	NS	Good	Good	Excellent
Swinging bucket	Inefficient	Good	Excellent	Good**
Zonal	NS	Excellent	Excellent	Good

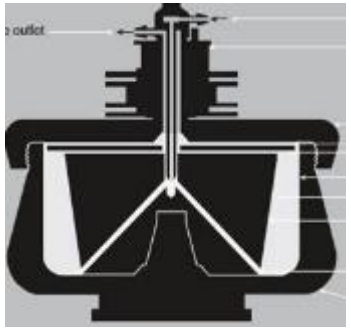
* good for macromolecules, poor for cells and organelles
**good for cells and organelles, caution needed if CsCl used
NS – not suitable

D. Rickwood, T.C. Ford and J. Steensgaard, 1994 Centrifugation Essential Data.

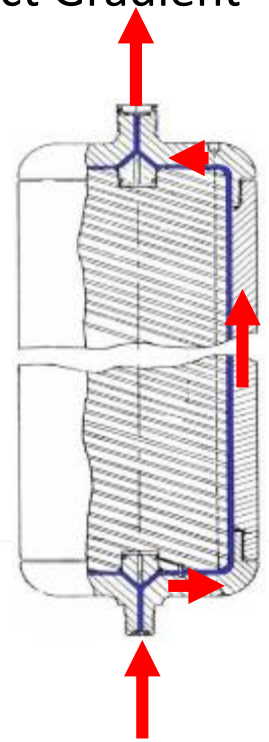
Scaling up ultracentrifugation usually operates in continuous flow mode

These rotors (both disc and tubular types) are not limited by their size as fluid continuously enters and leaves the rotor during high speed operation.

Principle: Load Gradient – Spin Rotor – Flow Product – Brake Rotor – Collect Gradient



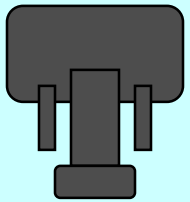
Parameter	CF32	AFH-KII
Shape	Disc type	Tubular
Speed max.	32 000 rpm	40 500rpm
Centrifugal force	102 000 xg	121 200 xg
Capacity	430 mL	3200 mL
K factor	42	29.7
Flow Path	Loop	Dual inlet
Scalable	No	Yes
Automated	Manual process	Automated



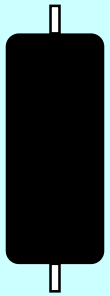
SEPARATION TECHNOLOGIES

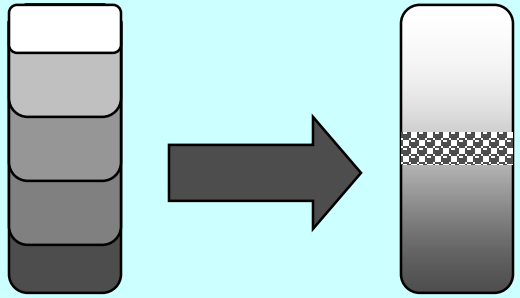
Transfer from small to large scale gradients requires that the separate tubes of a rotor are replaced by one tube of liquid which fills the rotor

Tube Rotor



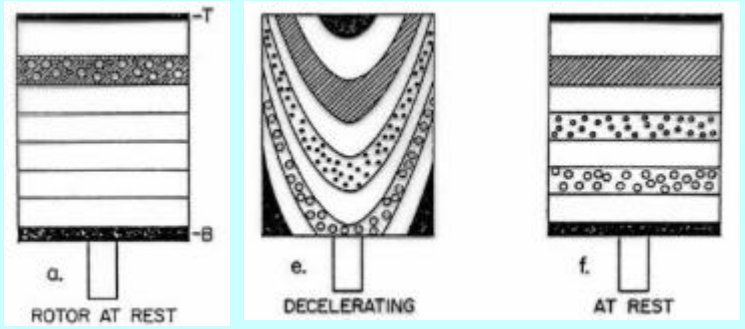
K3 Rotor





228 ml

12 ml (x20)

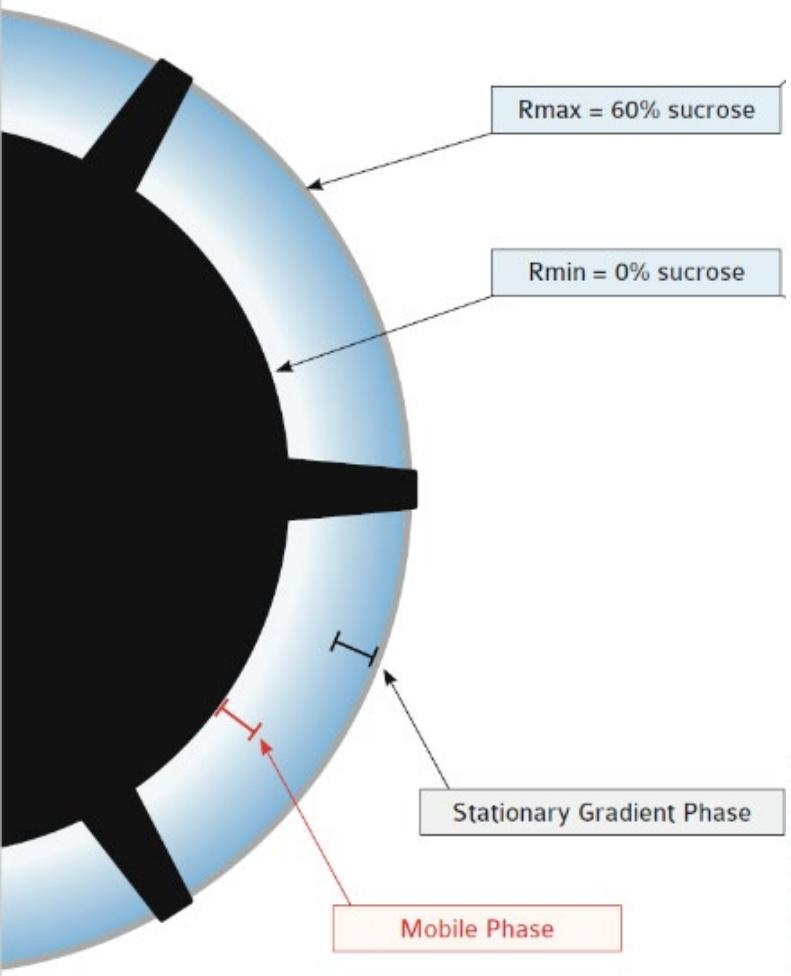


3200 ml

160 ml (x20)

The gradient itself reorients, within the rotor during the acceleration and deceleration phases

Scale up is made using the rotor technology of creating different volume separations within the same rotor



Maximum radius – must not change
Minimum radius – must not change
Gradient load density – must not change

Gradient load volumes – increases volumetrically on scale up
Product flow rate – increases volumetrically on scale up

Particle Range	700S
Flow / Batch	Continuous Flow
Rotor Type	K3 3200 PK3-1600 PK3-800 PK3-400 PX3-230 PX3-120
Flow Rate	28 L/h 14 L/h 7 L/h 3.6 L/h 2.0 L/h 1.0 L/h

SEPARATION TECHNOLOGIES

Alfa Wassermann ultracentrifuge system capacities

Promatix

	AW Promatix 1000®	PKII	KII
Typical Feed Flow	0.25 – 2 L/h	up to 15 L/h	up to 30 L/h
Rotor Size	PX3 – 120 mL PX3 – 230 mL	PK3 – 400 mL PK3 – 800 mL PK3/PK6 – 1600 mL	K3/K6 – 3200 mL *K10 – up to 8 Liters* *K5 – 8.4 Liters*
Batch Volume (5h run)	Up to 5 L	Up to 75 L	Up to 150 L
Max. Rotation Speed	35,000 rpm	40,500 rpm	40,500 rpm
Gravitational Forces	Up to 90,500xg	Up to 121,200xg	Up to 121,200xg
Scale Factor	27x scale down 14x scale down	8x scale down 4x scale down 2x scale down	1x scale

PKII

KII

SEPARATION TECHNOLOGIES

Scale up efficiency means that large volumes of material can be processed in a short time

Scale Volume	Process Volume	Rotor Volume	Run operations / days	Process 1 liter
Laboratory	23ml (10%)	38ml x 6 = 228ml	16 h (overnight)	44 runs
Laboratory Batch	160ml (10%)	1600ml	16 h (overnight)	6 runs
Laboratory Continuous flow	Up to 20 liters (5 L/h)*	430ml	8 h (day run)	12 min
AW PKII	Up to 25 liters (3.7 L/h)*	400ml	8 h (day run)	2 min
AW PKII	Up to 50 liters (7.5 L/h)*	800ml	8 h (day run)	2 min
AW PKII	Up to 100 liters (15 L/h)*	1600ml	8 h (day run)	2 min
AW KII	Up to 200 liters (30 L/h)*	3200ml	8 h (day run)	2 min

*dependent on particle size

AAV purification using continuous flow Iodixanol density gradient ultracentrifugation in the Promatix 1000

Bac-inCapX-inRep2

MOI = 10
X: any serotype

Bac-Gene of Interest

MOI = 5

Sf9

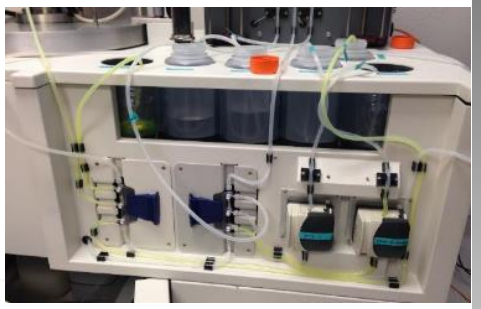
ESF921 (serum free)

3 days

Harvest cell pellet and prepare lysate

Continuous flow ultracentrifugation

Buffer exchange and qPCR

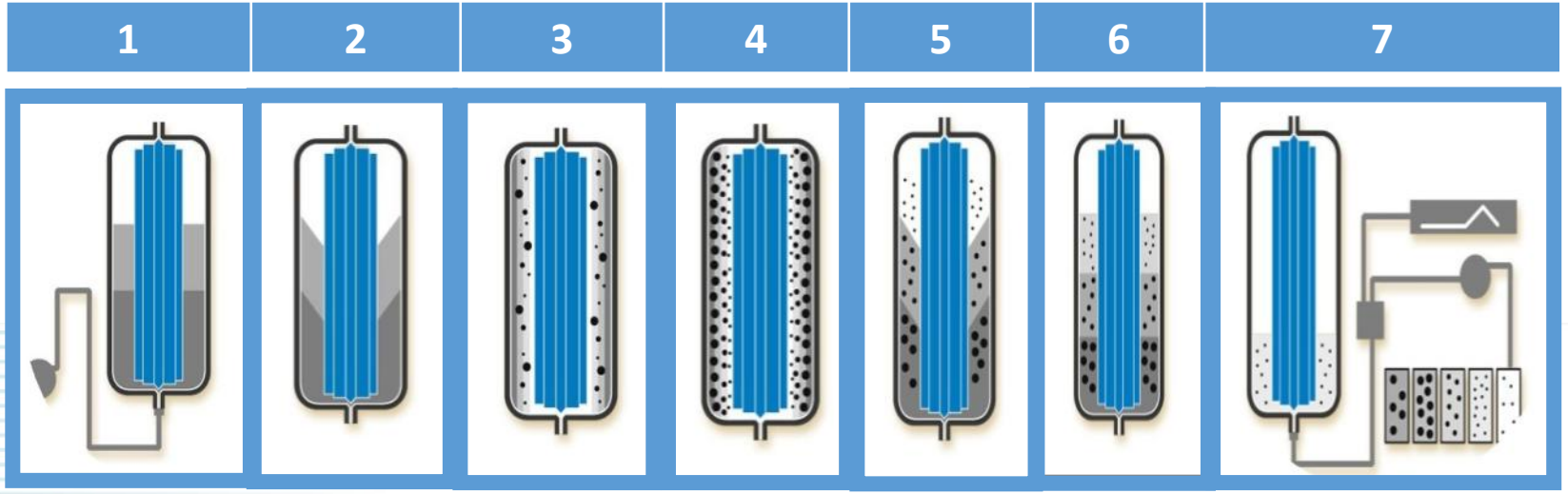


AAV and ultracentrifugation work by
Dr Haifeng Chen, Virovek Inc, Hayward,CA, USA

SEPARATION TECHNOLOGIES

Reorienting density gradient centrifugation using continuous flow

1. With a stationary rotor the AFH loads the gradient.
2. Slow acceleration reorients the gradient.
3. At 35 000 rpm flow sample into the rotor; vector is captured in the density gradient, waste flows out.
4. Isopycnic sedimentation occurs in zones where the gradient density equals a particle's buoyant density.
5. Slow deceleration reorients the gradient and retains the separation zones.
6. AFH collection and analysis.



AAV Purification Results

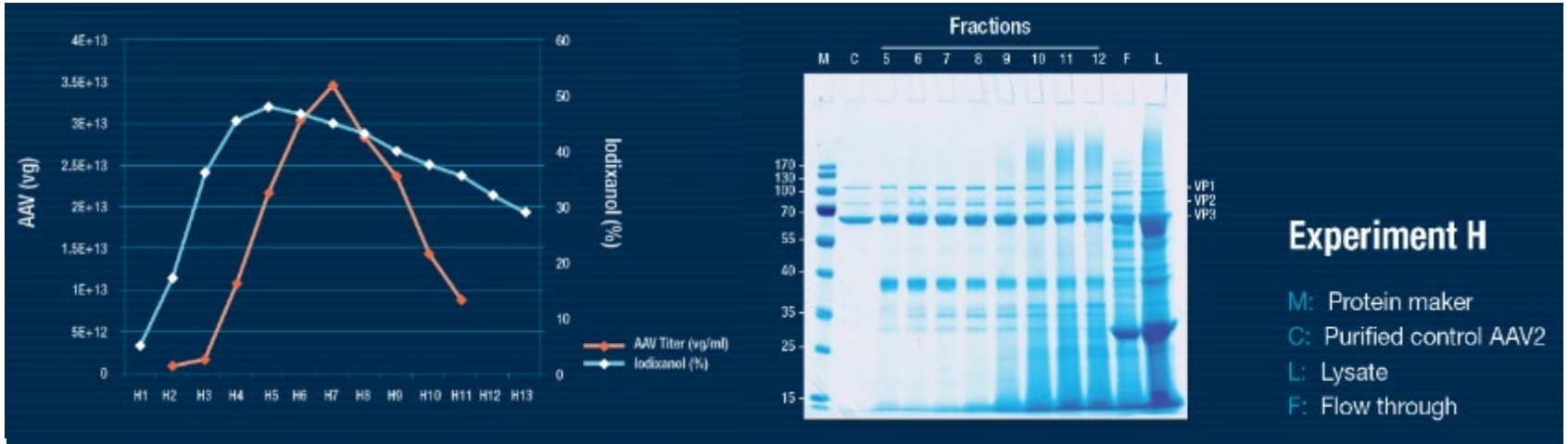


Fig. 4 Improvement of AAV recovery rate by adding sodium citrate to the iodixanol gradients and by using step gradients.

A 120-ml rotor was loaded with 25ml of 25% and 58ml of 50% iodixanol containing 100mM sodium citrate. After the speed reached 35,000rpm, a total of 95ml cleared cell lysate was loaded at 5ml/min continuously into the rotor. After centrifugation at 35,000rpm for 2 hours, the speed was slowly decreased, fractions collected, and AAV titer determined. The results indicate that AAV recovery was increased to over 50% (A) and that the AAV vectors were separated from the bulk cellular proteins as shown by the SDS-PAGE and SimplyBlue Staining (B).

Scale up of Iodixanol density gradients 27 fold using the AW scalable ultracentrifuges

Promatix 1000
R&D

PKII
Pilot Scale

KII
Manufacturing Scale

Fixed Parameters:
Maximum radius
Minimum radius
Gradient load density

Variable Parameters:
Gradient load volumes – increases volumetrically

Product flow rate – increases volumetrically

SEPARATION TECHNOLOGIES

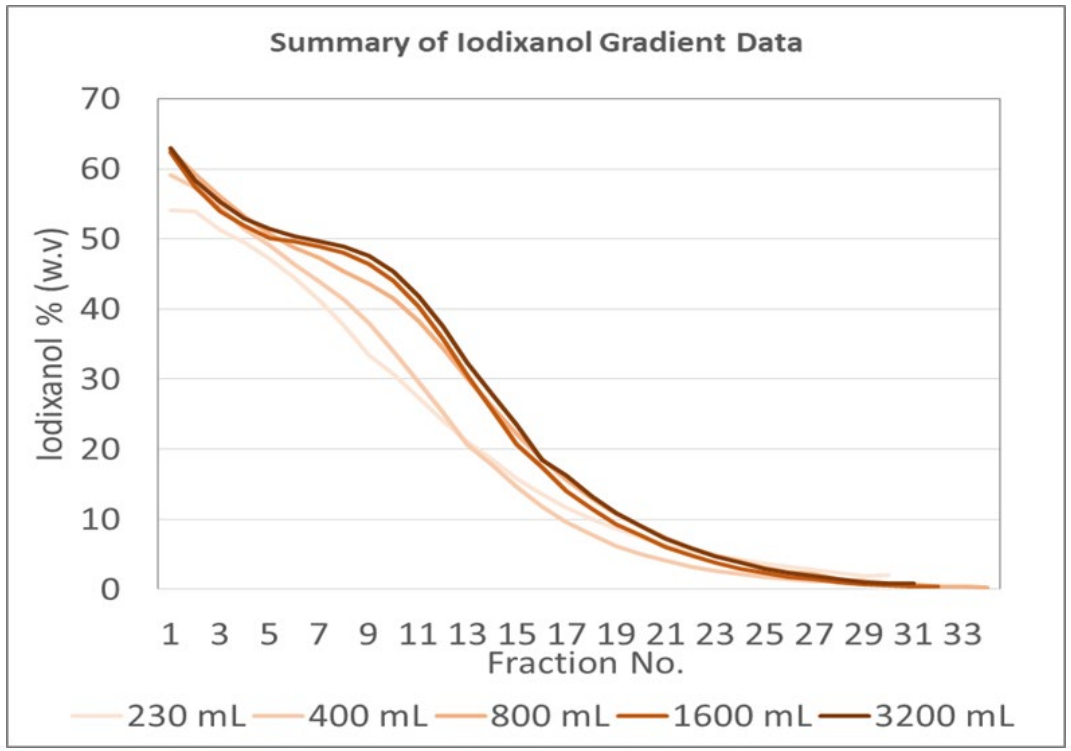
Scale up of Iodixanol density gradients 27 fold using the AW scalable ultracentrifuges

All systems: run at 35000rpm, 90 500xg, AAV residence time is 11.6 minutes, banding time is 2 hours.

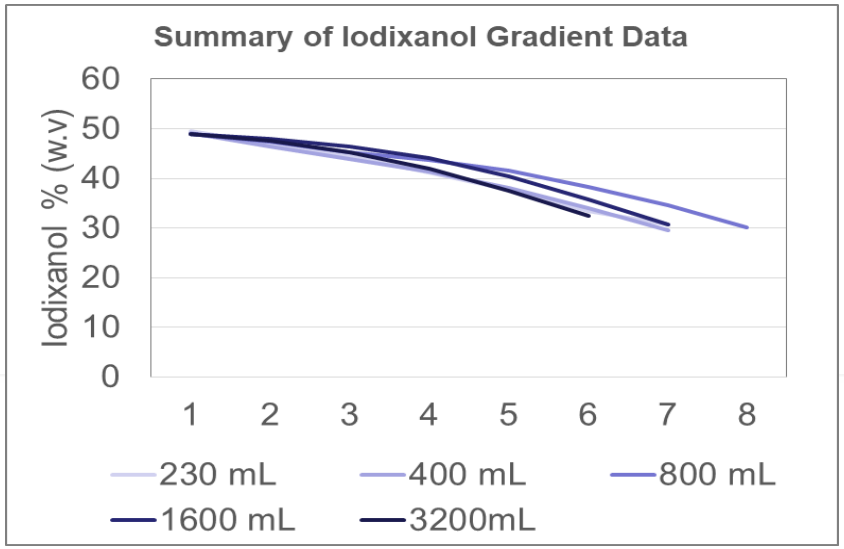
Core/Vol.	25% (w.v) Iodixanol	50% (w.v) Iodixanol	Sample Volume	Flow Rate
PX-120mL	20 mL	45 mL	80 mL	5 mL/min
PX-230mL	40 mL	90 mL	160 mL	10 mL/min
PK-400mL	70 mL	165 mL	280 mL	17 mL/min
PK-800mL	140 mL	330 mL	560 mL	34 mL/min
PK-1600mL	280 mL	660 mL	1120 mL	68 mL/min
KII-3200mL	560 mL	1320 mL	2240 mL	136 mL/min

SEPARATION TECHNOLOGIES

Iodixanol density gradients using continuous flow ultracentrifugation scale up in a predictable pattern



It was established form the work with the AAV vector that the separation zone of the AAV in the density gradient was between 32% and 48% Iodixanol. Using this information analysis of the separation zone within this zone was made.



SEPARATION TECHNOLOGIES

Conclusion

- Density Gradient ultracentrifugation is a scalable process for use in gene therapy production
- Linear scale up of Iodixanol density gradients is possible