Case study of continuous chromatography (MCSGP) on production for oligonucleotide

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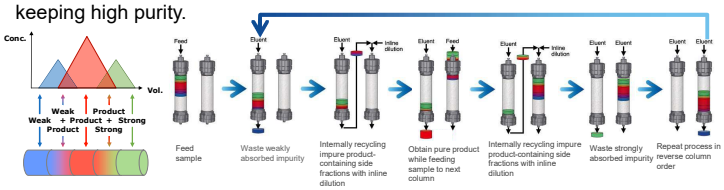
Introduction

An efficient purification is required in downstream process for production of oligonucleotide pharmaceuticals due to related impurities having similar chemical properties. While a conventional single-column purification faces a purity-yield trade-off challenge, a continuous chromatography process: MCSGP (**M**ulti-column **C**ounter-current **S**olvent **G**radient **P**urification) achieves both high purity and yield at the same time by recycling side-cut portions periodically. As results of MCSGP for oligonucleotide, the yield was improved 1.3-fold compared to its single column purification and purity-yield trade-off was overcome. Furthermore, an estimate of a 200 g production scale and a simulation of a manufacturing facility were conducted.


Contichrom® TWIN100

MCSGP

MCSGP is a purification process which two columns are used in continuously. Feed sample is separated through a column by solvent gradient to pure product (red), impurities (blue and green) and mixture of impurities/product (blue/red and red/green). In a conventional single-column purification, the mixtures which are not pure enough are wasted or collected for re-chromatography. On the other hand, MCSGP recycles these portions to the other column with in-line dilution, enabling to prevent the loss of precious target compound. In addition to recycled portions, new feed is injected with the same amount of pure product collected in the previous column. Repeating this cycle achieves high yield with keeping high purity.



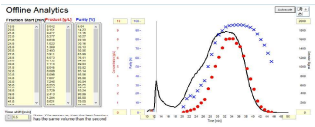
Sample

Classification	: Crude Model MOE-ASO 18mer, PS(full), 2'-MOE
Sequence	: 5'-TCACTTTTCATAATGCTGG-3'
Purity	: 75.6 %

Purification

MCSGP: Continuous Chromatography

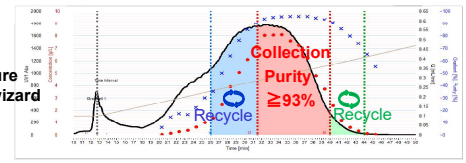
✔ MCSGP condition can be configurated from Batch fraction data using software incorporated in Contichrom® CUBE.



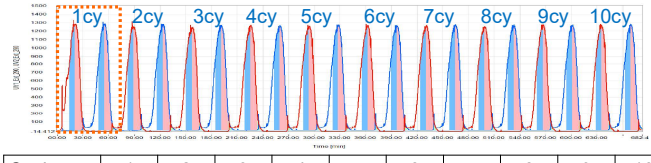
Column : BioPro IEX SmartSep Q (20 µm) 100 X 5 mm i. d.
Eluent : A) 20 mM Tris-HCl (pH 8)
B) 20 mM Tris-HCl (pH 8) containing 3M NaCl
15-75%B (12 CV)
Flow rate : 0.6 ml/min
Temperature : ambient
Detection : UV at 290 nm
Injection : net. 27 g/L-gel


Lab scale equipment Contichrom® CUBE

Guided procedure through MCSGP wizard



✔ 10cy-MCSGP was successfully conducted with keeping purity criterion ≥93%.
✔ After 2nd cycle, the product of each cycle was constantly obtained in high yield, 85-90%.

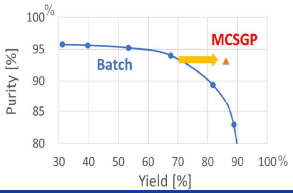


Cycle	1	2	3	4	5	6	7	8	9	10
Purity [%]	94.9	93.3	93.1	93.0	93.1	93.1	93.0	92.8	92.7	93.0
Yield [%]	72.7	88.6	88.0	88.0	89.4	89.5	89.4	89.5	88.0	88.5

Comparison with Batch

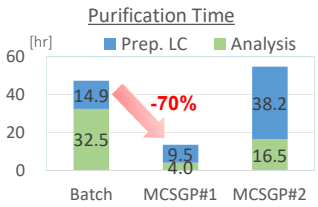
✔ The yield was improved by 1.3-fold compared to Batch.
✔ The trade-off between purity and recovery was overcome.

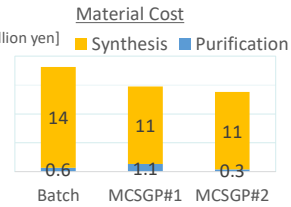
	Batch	MCSGP
Crude Purity [%]	75.6	75.6
Product Purity [%]	94.0	93.2
Yield [%]	66	X 1.3 86
Productivity [mg/hr]	33.5	X 1.6 52.4



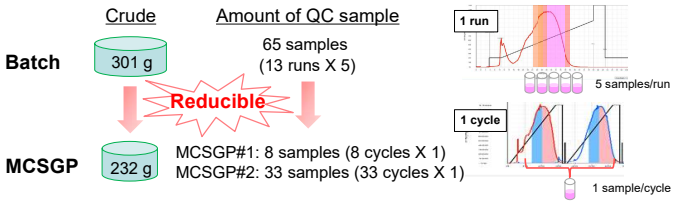
Estimate of 200 g production scale

✔ Based on the MSCGP study, the purification time and material cost were estimated. Two patterns of MCSGP (#1, #2, column sized difference) were simulated for comparison.
✔ MCSGP can reduce purification time by 70% and save the material cost due to decreased amount of crude material.
✔ Purification time of MCSGP using smaller columns (MCSGP#2) is on the same level as that of Batch. Downsizing a skid is available.



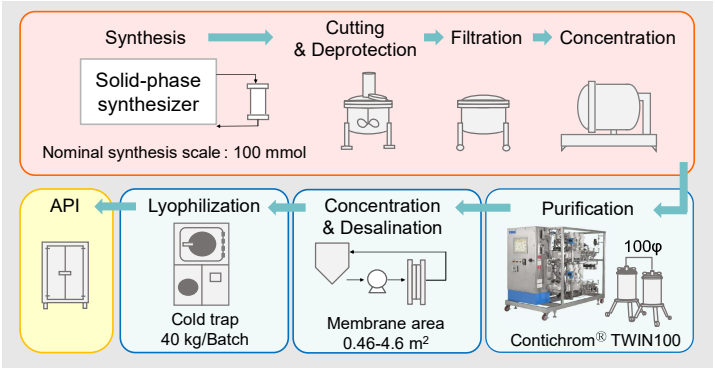


	Batch	MCSGP #1	MCSGP #2
Column size	100 X 100 mm i. d. (resin vol: 0.79 L)	100 X 100 mm i. d. x 2 (resin vol: 1.57 L)	100 X 50 mm i. d. x 2 (resin vol: 0.39 L)



Simulation of manufacturing facility using MCSGP

✔ 0.48 to 20 kg of API can be manufactured by the same equipment. A facility with MCSGP can produce API on a scale from development to commercial.



Process	Mfg amount	
	kg/week	kg/year
Synthesis	0.60	25.2
Purification	0.51	21.4
Concentration & Desalination	0.48	20.2*
Lyophilization		

* Max.80kg/year by 4 synthesizers without changing downstream skid

Conclusions

- MCSGP achieved high yield while maintaining purity criterion ≥93%. The yield was improved by 1.3-fold compared to Batch. A purity-yield trade-off was overcome.
- As an estimate on a 200 g production scale, MCSGP can reduce purification time by 70% and save the material cost due to decreased amount of crude material. Downsizing a skid by smoller columns is achievable.
- 0.48 to 20 kg of API can be manufactured by the same equipment. A facility with MCSGP can produce API on a scale from development to commercial.

Acknowledgment

✔ This poster was supported by AMED under Grant Number JP21ae0121022, JP21ae0121023 and JP21ae0121024 (Project leader: Satoshi Obika).
✔ A model antisense oligonucleotide therapeutics("Model MOE-ASO 18mer") was synthetized by KNC Laboratories Co.,Ltd.
✔ Simulation of manufacturing facility was supported by PeptiStar Inc. and CHIYODA X-ONE ENGINEERING.