Enhancing yield and productivity through the optimization of loading and surface area of CPG Solid Support

Shiva P Adhikari[1], Michael Greisen[1], Jan Schoenherr[1], Koby Ashizawa[1], Edward Sandoval[1], Songjun Xiao[1], David Hannah[1], Shireen Ahmed[1], John Tukianen[2], Liang Noinala[2], Rahul Talekar[2], Jun Shi[2], Carl Hitscherich Jr[2], Jim Lucie[2] and Daren Dick[1]*

[1]LGC Biosearch Technologies, [2]Alnylam Pharmaceuticals, *Corresponding author: daren.dick@lgcgroup.com

Introduction

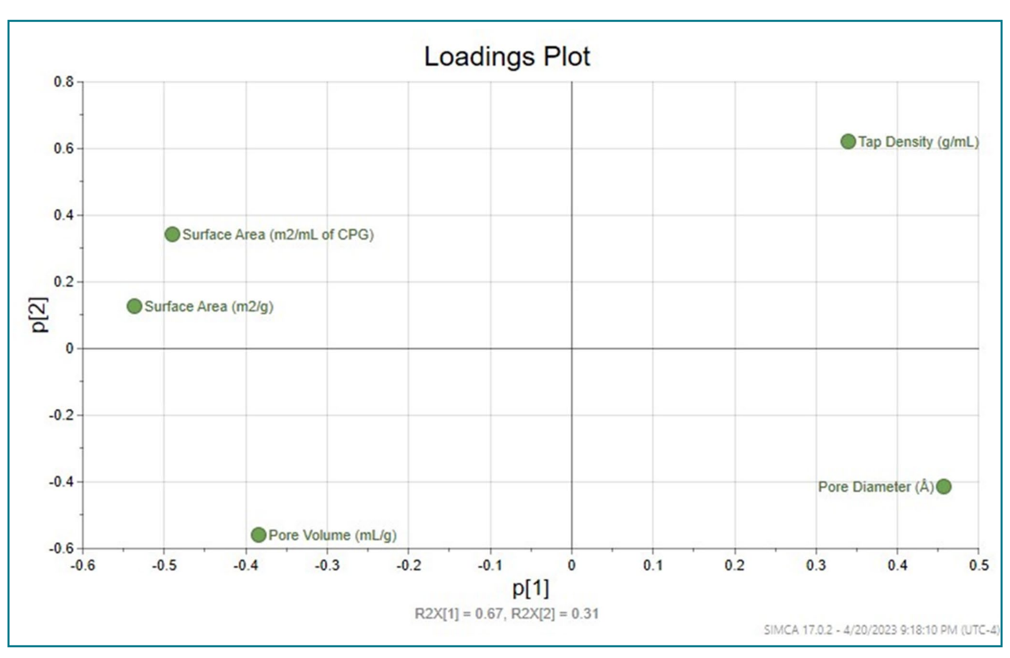
Historically, DMT-ligand loading of controlled pore glass (CPG) supports has been reported in terms of µmol/gram. While analyzing factors affecting performance of different CPG lots across a variety of pore diameters and oligonucleotide (oligo) types, a consistent observation has shown that higher loading levels and crude yields are strongly negatively correlated with surface area normalized loading (SANL), expressed in terms of µmol of DMT ligand per square meter of CPG surface area. In addition to reduced yields, we have observed increased prevalence of early coupling failures in some higher loaded CPGs. Together these observations have led to the working theory that below a critical SANL threshold, loading can be increased resulting in increased synthesis scales and productivity. However above that threshold, steric crowding at the surface results in reduced coupling efficiencies, particularly in early cycles, reducing yields and purities accordingly. Thus, to optimize CPG performance we need to identify this threshold and control loading in terms of µmol/m². In collaboration with Alnylam Pharmaceuticals and using a siRNA antisense strand (23mer, mixed 2'O-methyl/2'Fluoro RNA) as a model sequence, Biosearch Technologies has initiated a program to develop a CPG optimized for siRNA strand synthesis. The goals of the program are to (1) increase asset productivity in terms of yield and purity and (2) reduce cost per gram of the active pharmaceutical ingredient (API). This is being accomplished by tuning physical properties to maximize surface area and enable higher CPG loading, and by optimizing SANL ranges to consistently deliver the highest possible full-length product (FLP) yields. This poster describes initial proof of principle experiments and insights gained from early results of our efforts.

CPG physical properties

CPG physical property relationships

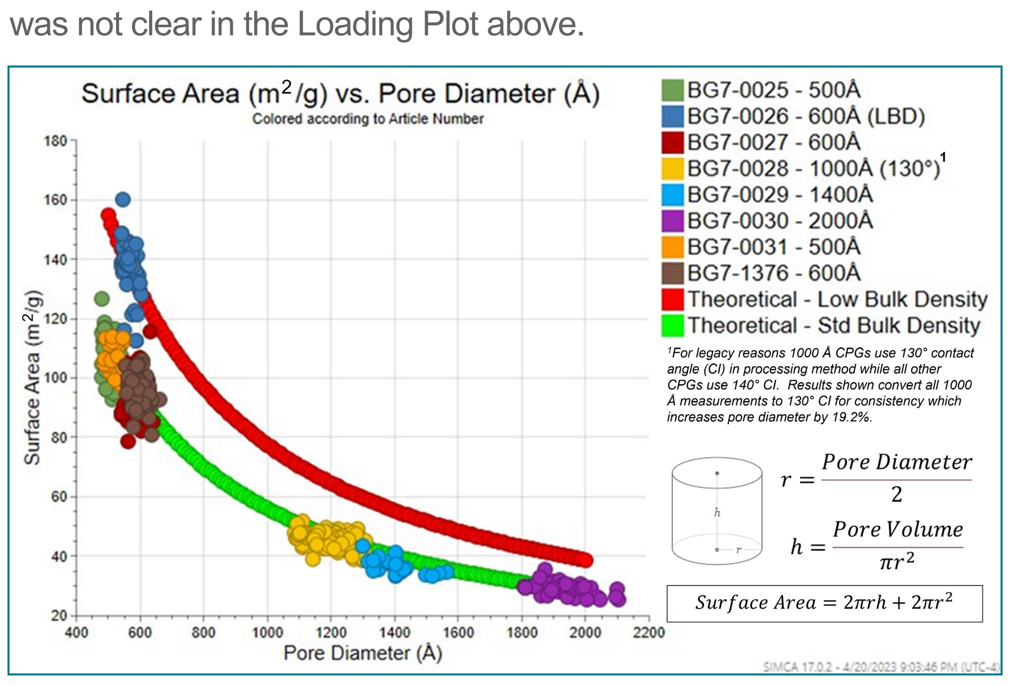
450 batches of CPG analyzed using SIMCA 17.0 software

- Inverse correlation observed: Pore Diameter vs Surface Area, Tap Density vs Pore Volume
- Negligible or no meaningful correlation between Tap Density or Pore Volume vs Pore Diameter



Surface area vs pore diameter

Negative correlation between surface area and density is illustrated when comparing surface area of low bulk density (LBD) and standard bulk density (SBD) CPGs of similar pore diameters. This relationship was not clear in the Loading Plot above.



Mercury intrusion porosimetry calculates surface area from measured pore diameter and volume at specific pressure points, assuming cylindrical pore shape.

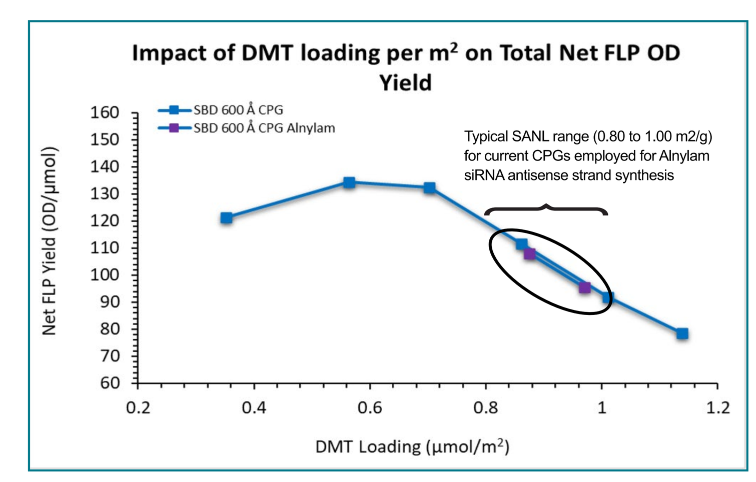
Conclusions of physical property analysis

- Smaller pore diameter and lower density CPGs maximizes surface area for loading.
- Lower density CPGs maximize pore volume.
- Pore volume is not dependent on pore diameter.

Identifying optimal loading range for current base CPG

Study on the effect of SANL DMT (µmol/m²) vs Net FLP Yield using a common amine CPG intermediate

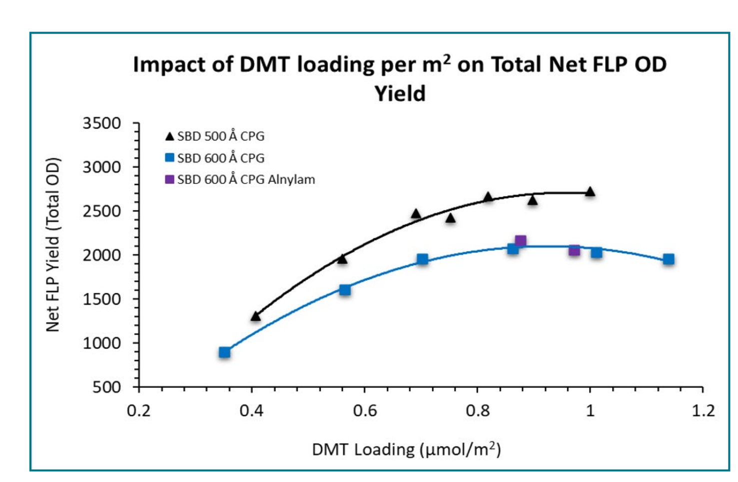
CPG Type	Surface Area, Hg (m^2/g)	DMT Loading µmol/g	DMT loading (µmol/m²)	% FLP	Gross Crude Yield (OD/µmol)	Net FLP Yield (OD FLP/µmol)	DMT Loading µmol/mL	Synthesis Scale (µmol)	Total Net FLP OD Yield
SBD 600	94	33	0.35	73.11	166.1	121.5	9.6	741	0.90×10^5
SBD 600	94	53	0.56	75.28	178.8	134.6	15.6	1199	1.61×10^5
SBD 600	94	66	0.70	75.32	175.9	132.5	19.3	1483	1.97×10^5
SBD 600	94	81	0.86	72.25	154.4	111.6	24.2	1863	2.08×10^5
SBD 600	94	95	1.01	68.85	133.7	92.1	28.7	2207	2.03×10^5
SBD 600	94	107	1.14	68.30	115.3	78.7	32.3	2486	1.96×10^5
SBD 600	96	84	0.88	72.09	150.0	108.1	26.0	2003	2.17×10^5
SBD 600	88	85	0.97	70.70	135.0	95.4	28.1	2158	2.06×10^5



- Consistent Net FLP Yield up to 0.70 µmol/m²
- Reduced Net FLP yields beyond loading levels 0.70 µmol/m²
- Elevated levels of early failures in higher loaded supports (see 'Synthesis failure comparison...')
- Excellent overlap with data produced by Alnylam for the same sequence on different CPG lots

Productivity enhancement by increasing surface area through tuning of pore diameter and density

CPG Type	Surface Area, Hg (m^2/g)	DMT Loading µmol/g	DMT loading (µmol/m²)	% FLP	Gross Crude Yield (OD/µmol)	Net FLP Yield (OD FLP/µmol)	DMT Loading µmol/mL	Synthesis Scale (µmol)	Total Net FLP OD Yield
LBD 500	165	67	0.41	71.70	151.8	108.8	15.8	1204	1.31×10^5
LBD 500	165	92	0.56	74.16	156.8	116.3	21.0	1688	1.96×10^5
LBD 500	165	114	0.69	74.56	157.4	117.4	27.7	2112	2.48×10^5
LBD 500	165	124	0.75	74.29	141.5	105.2	30.8	2312	2.43×10^5
LBD 500	165	135	0.82	73.70	142.8	105.2	33.5	2534	2.67×10^5
LBD 500	165	148	0.90	71.64	131.0	93.9	36.7	2801	2.63×10^5
LBD 500	165	165	1.00	71.31	121.3	86.5	41.9	3156	2.73×10^5



- Greater surface area leads to higher loading capacity while still achieving high quality synthesis.
- Low bulk density CPGs have lower packing densities but provide higher loading per mL of column volume.
- Higher loading capacity results in increased yield of FLP OD available for downstream purification.

Synthesis failure comparison by IP-RP-UPLC-MS analysis

SBD 600Å - IPRP Impurity Profile by DMT Loading

DMT Loading (µmol/g)	DMT Loading (µmol/m²)	N-22 → N-15	N-14 → N-10	N-9 → N-4	N-3 → N-1	FLP
		Summed % Area Normalized				
33	0.35	7.77	3.11	3.95	7.10	73.11
53	0.56	6.16	2.36	3.99	6.59	75.28
66	0.70	6.09	3.72	4.19	6.21	75.32
81	0.86	7.88	6.44	3.13	6.25	72.25
95	1.01	11.58	6.09	2.98	6.70	68.85
107	1.14	13.00	5.72	2.84	6.90	68.30

- Increasing DMT loading beyond 0.7 µmol/m² resulted in elevated quantities of early synthesis failures in both LBD 500 Å CPG and SBD 600 Å CPG.
- Later synthesis failures (N-9 → N-1) are less impacted by DMT loading.

LBD 500Å - IPRP Impurity Profile by DMT Loading

DMT Loading (µmol/g)	DMT Loading (µmol/m²)	N-22 → N-15	N-14 → N-10	N-9 → N-4	N-3 → N-1	FLP
		Summed % Area Normalized				
67	0.41	9.38	2.34	2.54	8.50	71.70
92	0.56	7.00	3.53	2.42	7.32	74.16
114	0.69	6.81	4.20	2.69	6.68	74.56
124	0.75	7.20	4.47	2.77	6.46	74.29
135	0.82	7.71	4.99	2.83	6.29	73.70
148	0.90	8.25	6.10	2.87	6.22	71.64
165	1.00	10.13	4.71	2.42	6.27	71.31

- Impurity profile of current product (SBD 600 Å, 81 µmol/g) and our best current recommendation (LBD 500 Å, 114 µmol/g), were very similar with no new impurities in 600 Å CPG.
- We observed a slight increase in N-1 impurities in the LBD 500 Å CPG vs the SBD 600 Å CPG that will require further study.

Experimental design

- Synthesis of Alnylam siRNA antisense strand
- Instrumentation: Cytiva AKTA OP-100 Synthesizer; FL-35 column, 8.0 cm bed height
- 600 Å CPG loaded at 80-90 µmol/gram
- Testing theory: SANL is crucial to quality
- To find the **optimal loading level**, single lot of 600 Å amine CPG was loaded across a wide SANL range.
- To test whether **optimal SANL is independent of pore diameter and surface area**, loading curve on 500 Å LBD CPG with high surface area was repeated.

Conclusions

- Proof of principle study delivered a 19% increase in total FLP OD yield moving from current product to LBD 500 Å CPG loaded at optimal SANL, which show potential reduction in COGs.
- Optimal CPG loading per surface area is observed around 0.70 µmol/m²
- Reducing SANL for current product into optimal range will improve efficiency but reduce synthesis scales.
- Synthesis scales can be recovered or even improved by tuning pore diameter and density.

Next steps

- Optimize crude yield of LBD 500 Å CPGs to match that of 600 Å SBD CPGs through native and amine CPG intermediate optimization.
- Utilize DOE support to explore design space and establish a new specification for CPG optimized for Alnylam siRNA antisense strand synthesis.

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