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Surface functionalized nylon capillary-channeled polymer (C-CP) fibers for protein ion-exchange separations

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What are C-CP fiber columns ?

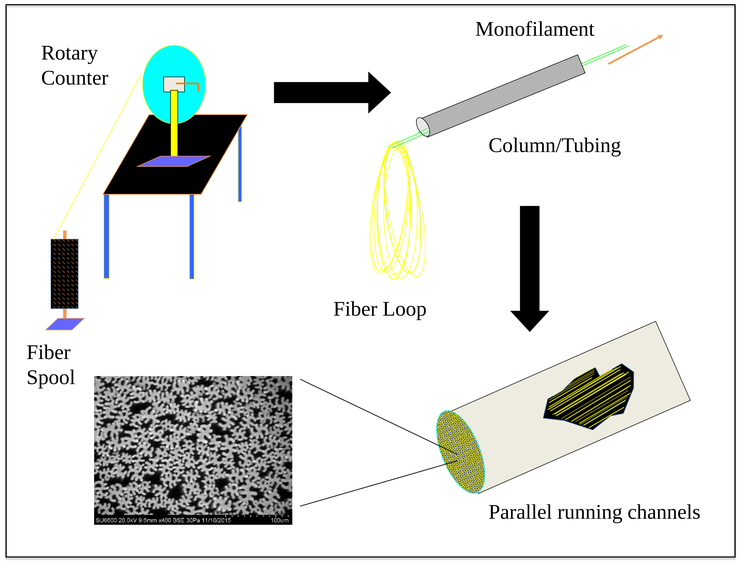
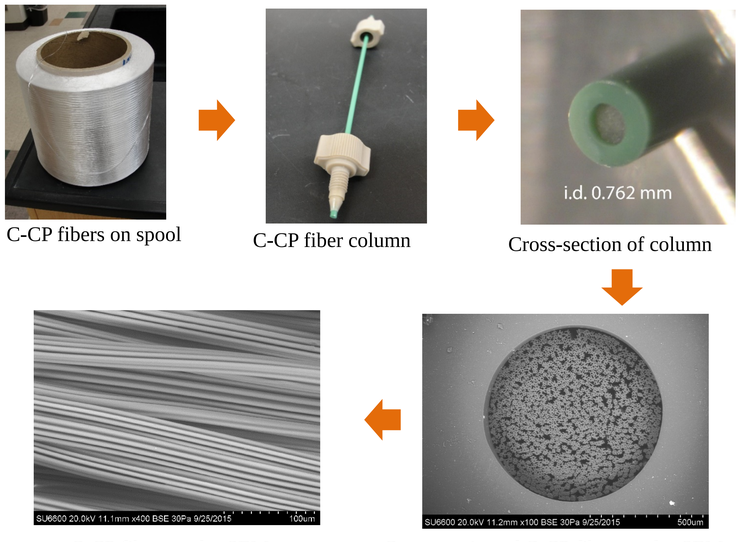


Figure 1. C-CP fiber column construction process

C-CP fibers are made from melt extrusion of commonly used polymers (e. g. PP, PET and nylon). There are 8 capillary channels on C-CP fiber which greatly increases the surface area and fluid transportation. C-CP columns are packed by pulling fibers through PEEK tubing.

Virtues of C-CP fiber column:

- Highly permeable (a 200 mm×2.1 mm i.d. C-CP column can be run at **10 mL min^{-1}** flow rate with back-pressure **<1500 psi**)
- Fast protein purification at high solvent flow rates
- **Low cost**

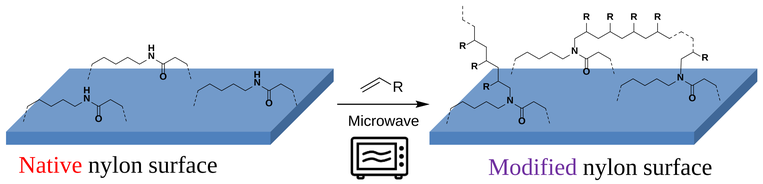


C-CP fibers on spool | C-CP fiber column | Cross-section of column

C-CP fibers under SEM | Cross-section of C-CP fibers under SEM

Nylon C-CP fiber Surface Modification

Native nylon C-CP fibers have a very low density (~20 μmol g^{-1}) of carboxylic acid groups that limits its performance on protein separations. To overcome this challenge, surface modification was done on native nylon fibers by grafting monomer ligands (acrylics) via microwave-assisted free radical polymerization.



Native nylon surface | Modified nylon surface

- **Simple modification:** Modification is fast (~10 min) and low-cost using a domestic microwave oven
- **High ligand density of modified fibers:** –COOH or –SO_3H ligands were grafted on native C-CP fibers in up to 500 μmol g^{-1} density
- **Versatile modification:** Different types of acrylic monomers are industrially available.

Protein Separations on C-CP fibers

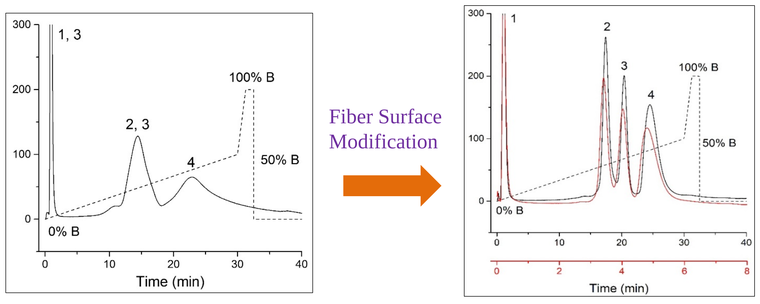


Figure 2. Protein separations comparison on **nylon-C-CP fiber** column before and after surface modification

- Proteins: 1. myoglobin, 2. α-chymotrypsinogen A, 3. cytochrome C, 4. lysozyme
- Column size: **200 mm length × 0.762 mm**.i.d.
- Mobile phase A: 20 mM phosphate pH 6.5, Mobile phase B: 1.0 M NaCl in A
- Flow rate: 0.1 mL min^{-1} (Black line), 0.5 mL min^{-1} (Red line)

Before surface modification

- Very poor protein ion-exchange separation on native fibers
- Co-elution of proteins

After surface modification

- **(Black line)**: Improved ion-exchange separation on modified column (Flow rate: 0.1 mL min^{-1})
- **(Red line):** Ion-exchange separation at a 5× increase flow rate (Flow rate: 0.5 mL min^{-1})
- Increase of flow rate by **5×** does NOT impair the separation resolution
- Decrease of total separation time from 30 min to 6 min

Protein Loading/Elution

Table 1. Dynamic binding capacity (DBC) of lysozyme on nylon C-CP fiber columns at different protein loading concentrations.

Loading Concentration (mg mL^{-1})	Native Nylon DBC (mg g^{-1})	Modified Nylon DBC (mg g^{-1})
0.05	0.63±0.44	19.13±0.18
0.1	1.81±0.02	20.11±0.02
0.2	0.87±0.19	20.56±0.01
0.4	0.81±0.27	21.07±0.38
0.6	1.24±0.47	22.22±0.03
0.8	0.94±0.67	22.28±0.04
1.0	1.66±0.10	22.60±0.89

Figure 3. Consistency of lysozyme loading and elution

- 10 lysozyme loading and elution chromatograms. No column regeneration was carried out between runs
- Excellent lysozyme recovery on modified nylon column
- **RSD of binding capacity = 0.3% (n=10)**

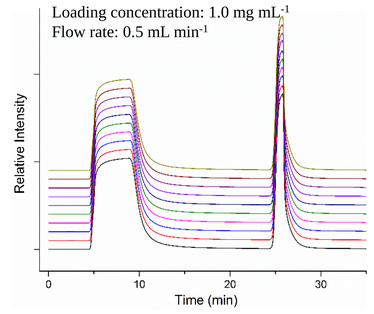
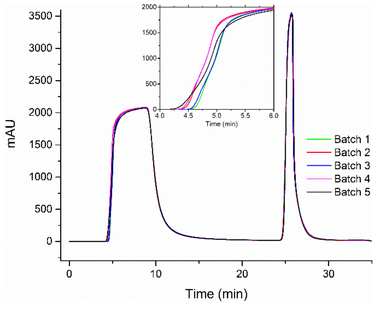


Figure 4. Column to column consistency

- Negligible variations in breakthrough curves
- Batch-to-batch reproducibility of the modification could be improved by using more controllable microwave instruments
- **RSD of binding capacity = 3% (n=5)**



Conclusions

Microwave-assisted grafting polymerization largely improved the performance of the nylon C-CP fiber packed column in protein separations. Applying this simple but versatile modification method on nylon C-CP fibers offers cost-efficient LC stationary phase that are capable of fast protein separations and high-throughput protein processing.

Acknowledgments

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References

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