BEYOND HIGH PURITY:
WHY ULTRAPURE EDC IS KEY FOR SUCCESSFUL AMINE COUPLING IN SPR

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Since its introduction in the early 1990s, amine coupling via EDC/NHS has become the gold standard for immobilizing amine-containing ligands on SPR sensor chips. This method enables covalent binding of ligands to preactivated polycarboxylate sensor coatings—such as CMD or HC hydrogels—resulting in excellent chemical stability and high coupling yields [1–3].

Recently, however, amine coupling has shown alarming signs of unreliability. Many SPR users, including experienced practitioners, now report low immobilization densities, and in some cases, complete failure. These issues are attributable to insufficient EDC quality from certain chemical suppliers; new findings reveal that such quality problems are more prevalent than previously known. Recognizing that EDC quality has a significant impact on immobilization success, and given the growing evidence of insufficient quality from many suppliers, we have prepared this technical note to explicitly address this issue, covering the following key aspects:

- Demonstrating how impure EDC affects typical SPR immobilization patterns.
- Performance benchmarks for leading suppliers of “high-purity” EDC.
- Explanation of the severe effects of trace impurities on the coupling process.
- Recommendations about which EDC preparations to trust.

In Shape or Out of Shape – A Closer Look at SPR Curves to Detect EDC Quality

The SPR sensorgram of a polycarboxylate sensor chip exhibits a distinctive pattern during EDC/NHS coupling. Beginning from a stable baseline, the injection of the EDC/NHS solution causes a sharp signal increase of several thousand RU (µRIU), similar to a high-salt buffer shift. This signal stabilizes within minutes, then drops to a level a few hundred RU above the original baseline after injection of the activation buffer. Ligand preconcentration subsequently leads to a strong, gradual signal increase, stopping with the ethanolamine quenching step. The final baseline signal reflects the yield of ligand immobilization.

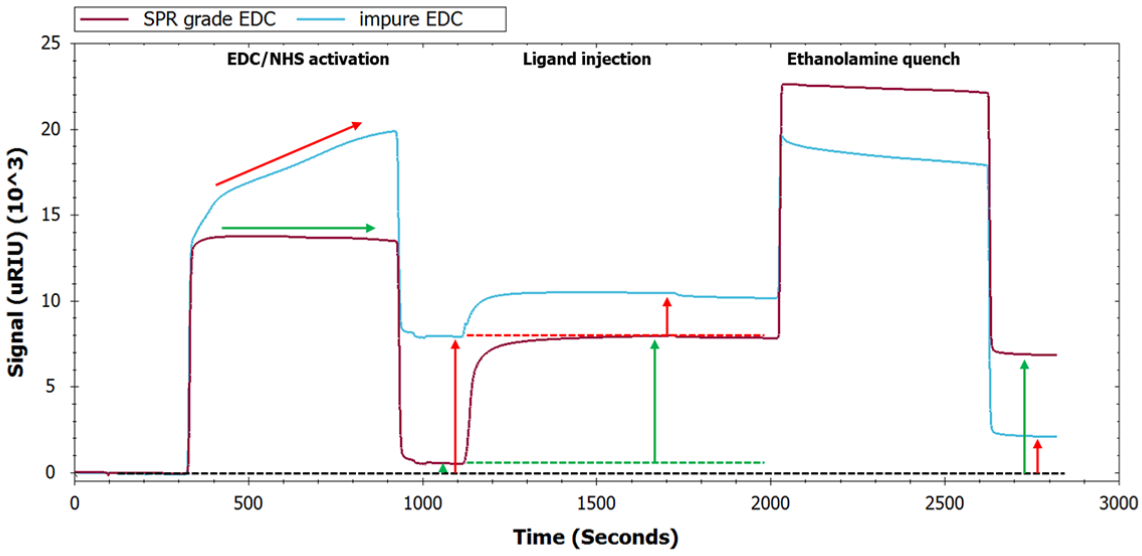

Fig. 1: Overlay of two typical SPR sensorgrams showing the amine coupling of bovine serum albumin in preconcentration conditions on an HC30M sensor chip using 260 mM (5%) EDC. The red profile depicts the typical SPR signal of a successful immobilization using SPR-grade EDC, while the blue profile illustrates the characteristic shape of an amine coupling when the EDC purity is insufficient.

In contrast, the SPR profile deviates significantly when using impure EDC. Starting from a stable baseline, the EDC/NHS injection produces a pronounced signal increase that continues rising throughout the injection period. Following activation, the baseline does not return to the expected level; instead, it shifts upward by several thousand RU. Protein preconcentration within the polycarboxylate matrix then fails, and after the ethanolamine quench, the SPR signal drops below the post-activation level. Though minimal protein coupling may occasionally occur, immobilization levels typically fall far below expectations, resulting in insufficient performance for most applications.

In Fig. 1, two SPR sensorgrams illustrate the typical steps of amine coupling. One immobilization was conducted with SPR-grade EDC (red), while the other used EDC of insufficient quality (blue). The outcome underscores the importance of using ultrapure EDC for consistent and reliable immobilization; impure reagents result in wasted time, resources, and compromised sensor performance.

When "High Purity" Isn't Enough

It should be emphasized that immobilization failure during amine coupling can still occur even with "high purity" EDC. Often sold with purity specification ≥99%, EDC is not typically suspected as the cause of failure—especially by experienced SPR users who handle it rigorously according to the storage instructions. Additionally, impurities, though visually inconspicuous, can be heterogeneously distributed, meaning that chance can sometimes dictate whether an amine coupling succeeds or fails. This makes it particularly challenging for SPR users to trace errors back to the coupling reagent.

To address EDC-related immobilization issues, we conducted benchmarking of EDC across well-known chemical suppliers commonly used by the SPR community. Our goal was to provide customers with clear information about which EDC products are reliable for SPR and which may lead to inconsistent results. All EDC products included in the study represent the highest available quality (Table 1).

Table 1: Overview of the EDC–HCl products that were considered in quality benchmarking.

Supplier	Product code	Purity	Lot number
XanTec	EDCHCL5G	SPR Grade	0724.a
Cytiva®[1]	BR100050	n. a.	36033
Thermo Fisher® (Pierce™)	PG82079	Pierce™ Premium Grade	ZI401280
Sigma Aldrich®	E1769	BioXtra, Premium grade	BCCL2739
Roth®	2156.1	≥99%	34273747

[1] Part of Amine Coupling kit.

To evaluate the qualities of the different EDC products, two test systems were applied. In the first, widely used HC30M sensor chips were activated with MES-buffered sulfo-NHS and EDC concentrations ranging from 5 to 260 mM (0.1% to 5%), with BSA serving as the model protein for immobilization. In the second system, typical immobilization conditions for CMD coatings were applied with four different proteins using CMD200L chips, the XanTec equivalent of Cytiva CM5 chips. All EDC samples were stored dry with desiccant at −20°C until use to rigorously limit hydrolytic decay.

In Fig. 2, the results of the evaluation round on HC30M sensor chips are presented. At a relatively low EDC concentration (26 mM), the EDC from all sources performed satisfactorily and allowed high immobilization densities, between 5700 and 9700 µRIU. However, at higher EDC concentrations (156 and 260 mM), coupling yields dropped significantly. This effect was so pronounced that the EDC from Sigma Aldrich® and Roth® appeared to result in complete immobilization failure in the highest-concentration conditions. EDC from Cytiva® and Thermo Fisher® still functioned to some extent, although with an 80% decrease in yield. In contrast, SPR-grade EDC from XanTec consistently provided reliable immobilization levels, even at high concentrations (156 and 260 mM).

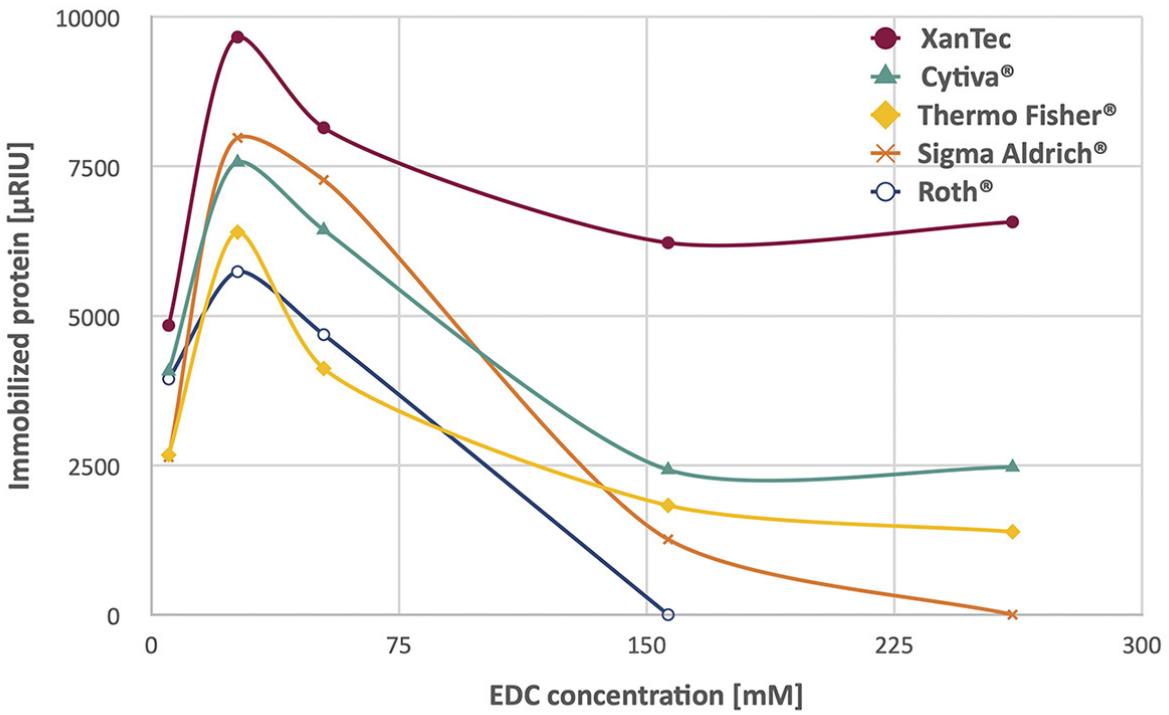

Fig. 2: Immobilization yield of BSA on XanTec HC30M sensor chips using EDC from various suppliers. The sensor surfaces were activated for 10 min with varying EDC concentrations (from 5 to 260 mM) in MES-buffered sulfo-NHS solution, followed by a 10-min injection of BSA (100 µg/mL in 5 mM NaAc, pH 5.0). Loosely bound BSA was removed by a 10-min ethanolamine quenching step. Experiments were conducted using a Reichert 4SPR. The lines in the graphs are provided as visual guides.

These results indicate that while successful activation could, in principle, be achieved with all EDC sources tested, the robustness of the immobilization process varied greatly. High EDC concentrations, in particular, proved to be problematic for all EDC suppliers except XanTec. This creates an added challenge for the experimenter to select and apply the correct EDC concentration.

In the second experiment, various model proteins were immobilized on a XanTec CMD200L sensor chip using a Cytiva T200 SPR instrument. Immobilization followed the standard amine coupling procedure defined by the software with a final EDC concentration of 200 mM. As shown in Fig. 3, EDC from Cytiva and XanTec performed comparably well for all proteins tested, although Protein AG showed relatively low immobilization densities, likely due to the acidic pH of the preconcentration buffer. In contrast, the other three EDC variants showed a significant performance drop, although EDC from Thermo Fisher still provided an acceptable immobilization level for BSA and bovine carbonic anhydrase II. EDC from Sigma and Roth led to complete immobilization failure for all model proteins except BSA. These results closely align with previous findings using HC30M sensor chips, underscoring the importance of ultrapure EDC for reliable immobilization outcomes.

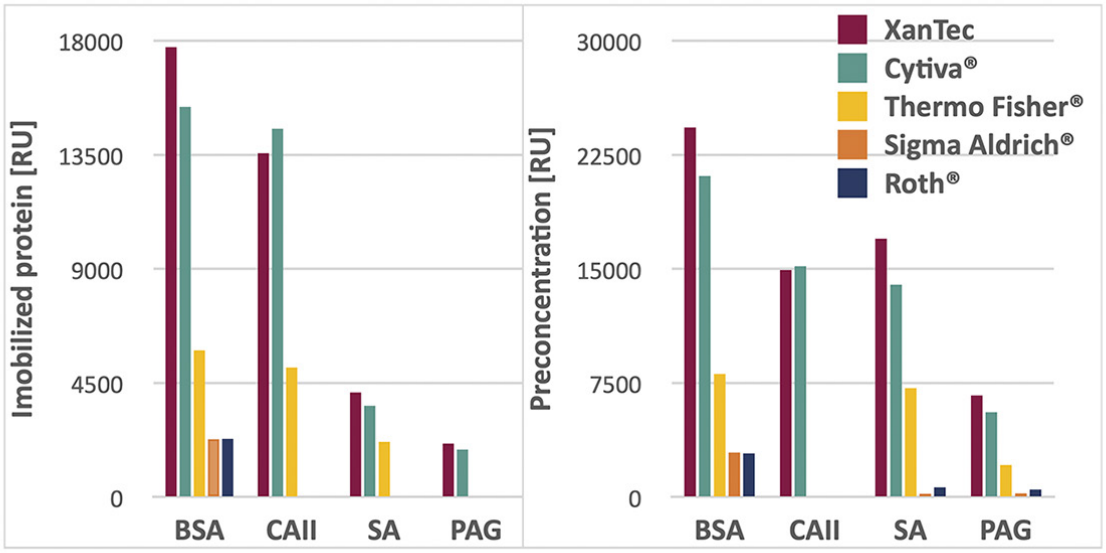

Fig. 3: Immobilization yield and maximum preconcentration level of four model proteins on XanTec CMD200L sensor chips (XanTec's CM5 equivalent) using EDC from various suppliers. The sensor chip was activated for 7 min with 200 mM EDC in MES/NHS buffer, pH 5.0. Proteins (100 µg/mL bovine serum albumin [BSA] in 5 mM NaAc, pH 5.0; 50 µg/mL streptavidin [SA] in 5 mM NaAc, pH 5.0; 50 µg/mL bovine carbonic anhydrase II [CAII] in 5 mM NaAc, pH 4.5; 50 µg/mL Protein AG [PAG] in 5 mM NaAc, pH 4.0) were allowed to preconcentrate for 7 min after activation. Ethanolamine (1 M, pH 8.5) was used to quench remaining NHS ester and remove loosely bound protein.

The Need for SPR-grade EDC

The experimental results above demonstrate that terms like "premium grade," "high purity," "bioXtra," or even purity levels ≥99% are insufficient indicators of the suitability of EDC for SPR applications. Even the highest purities fall short when compared with EDC from suppliers experienced in SPR. To clarify, we do not believe that EDC which underperforms in SPR is of poor quality overall. Rather, EDC for SPR applications must meet specific requirements that are often unmet by apparently high-quality offerings from most chemical suppliers. So, why is "high purity" often not pure enough for SPR?

The issue becomes clear when the number of functional groups on typical polycarboxylate coatings is compared with the proportion of impurities in the activation buffer—even trace impurities in low mass fractions can represent a large excess relative to the carboxyl groups on the sensor chip. For instance, an impurity level of 0.01% w/w of 3-(dimethylamino)propylamine dihydrochloride—an asymmetric diamine used in EDC manufacturing—represents an excess of ≈75 mole equivalents compared with the available carboxyl groups on typical SPR chips (see Supplementary calculations).

Furthermore, our investigations have shown that possible trace impurities often appear along with additional contaminants, which interact with SPR sensor coatings in the same way as polycations would do. Such impurities accumulate electrostatically within the hydrogel matrix during the EDC/NHS activation step, obstructing protein preconcentration (see Fig. 1). Additionally, primary and secondary amino groups on contaminants readily react with activated carboxyl groups on the sensor chip, inactivating them for ligand coupling. While such impurities may compromise SPR immobilization, standard EDC quality control rarely identifies these small amounts as problematic. In fairness, EDC with this purity will likely perform well in peptide synthesis or solution-based biochemical crosslinking procedures.

For SPR, however, the quality of EDC must exceed typical purity levels. Ensuring that the EDC supplier understands the unique requirements of SPR and provides consistently reliable quality is essential for successful use of the product in SPR applications. Because most chemical suppliers are not aware of SPR-related purity requirements, SPR users can never be sure about the performance of their freshly purchased EDC (even if a previous EDC batch from the same source performed well). To meet the need for a consistent supply of ultrapure EDC, XanTec has recently developed a rigorous purification process yielding a special SPR-grade EDC. Starting with already highly pure EDC, it is further purified in a multistage process that eliminates all possibly harmful trace contaminants. This SPR-grade EDC ensures maximum performance and reproducibility in SPR-based amine coupling, even at suboptimal EDC concentrations.

Conclusions

This brief technical communication has demonstrated that selecting the appropriate EDC source is crucial for the success or failure of SPR-based amine coupling. To avoid wasting time and nerve-racking troubleshooting, we recommend the following:

- **Use Reliable EDC Sources:** Whenever possible, source EDC from suppliers with a strong background in SPR who understand its specific quality requirements. If unsure, request SPR performance verification from the supplier.
- **Establish Internal Quality Control:** If the supplier cannot guarantee SPR performance, set up a robust internal QC test using a well-known model system to verify the suitability of the EDC for SPR applications. Note, however, that EDC contamination can be distributed heterogeneously.
- **Identify Poor-quality EDC Patterns:** Watch for SPR patterns that suggest low-quality EDC. A notably high signal increase following EDC/NHS activation, especially at high concentrations, is a key indicator of problematic EDC. If subsequent ligand immobilization fails despite successful preconcentration scouting, replace the EDC immediately.
- **Remember:** Contaminated EDC is one of the leading causes of failure in EDC/NHS-based ligand immobilization!

If you've recently faced issues with EDC/NHS-based amine coupling, we invite you to reach out to us at info@xantec.com. Share how impure EDC has hindered your experiments, and explore how our SPR-grade EDC can give your SPR experiments a boost.

Literature

1. *Fischer, M. J. (2010). Amine coupling through EDC/NHS: a practical approach. Surface Plasmon Resonance: Methods and Protocols, 55–73.*
2. *Johnsson, B., Löfås, S., & Lindquist, G. (1991). Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors. Analytical Biochemistry, 198, 268–277.*
3. *Cooper, M. A. (2003). Label-free screening of bio-molecular interactions. Analytical and Bioanalytical Chemistry, 377, 834–842.*
4. *Biacore T200 Instrument Handbook. Cytiva (formerly GE Healthcare), 28-9768-63 Edition AC, 02/2013.*

Supplementary calculations

Estimating the molar excess of 0.01% w/w trace impurity (3-(dimethylamino)propylamine dihydrochloride†) in EDC when used in standard immobilization conditions on a 3D CMD sensor chip:

1. Sensor Chip Carboxyl Groups in the Flow Cell
   - Approximate carboxyl group density of the sensor chip ≈ 3 nmol/cm²
   - Flow cell contact area [4]: 1.5 mm²

   3 nmol/cm² × 0.015 cm² = 0.045 nmol
2. Total EDC Passing Over the Surface
   - EDC concentration: 200 mM
   - Flow cell volume: 150 µL

   200 mM × 0.15 mL = 30 µmol (or 5.8 mg)
3. Amount of Impurity at 0.01% Mass Fraction

   5.8 mg × 0.01% / 175 g/mol = 3.3 nmol
4. Ratio of Impurity to Carboxyl Groups

   3.3 nmol/0.045 nmol = 74

†Exemplary of a potential trace contaminant that has a molecular weight of 175 Da and carries two positive charges in physiological conditions due to its two amine groups.

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