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New adsorbers for the removal of genotoxic impurities from active pharmaceutical ingredients

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New Adsorbers for the Removal of Genotoxic Impurities from Active Pharmaceutical Ingredients

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Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Portugal

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Outline

1. Introduction
2. Main Goal
3. Results and Discussion
4. Concluding Remarks
5. Acknowledgements

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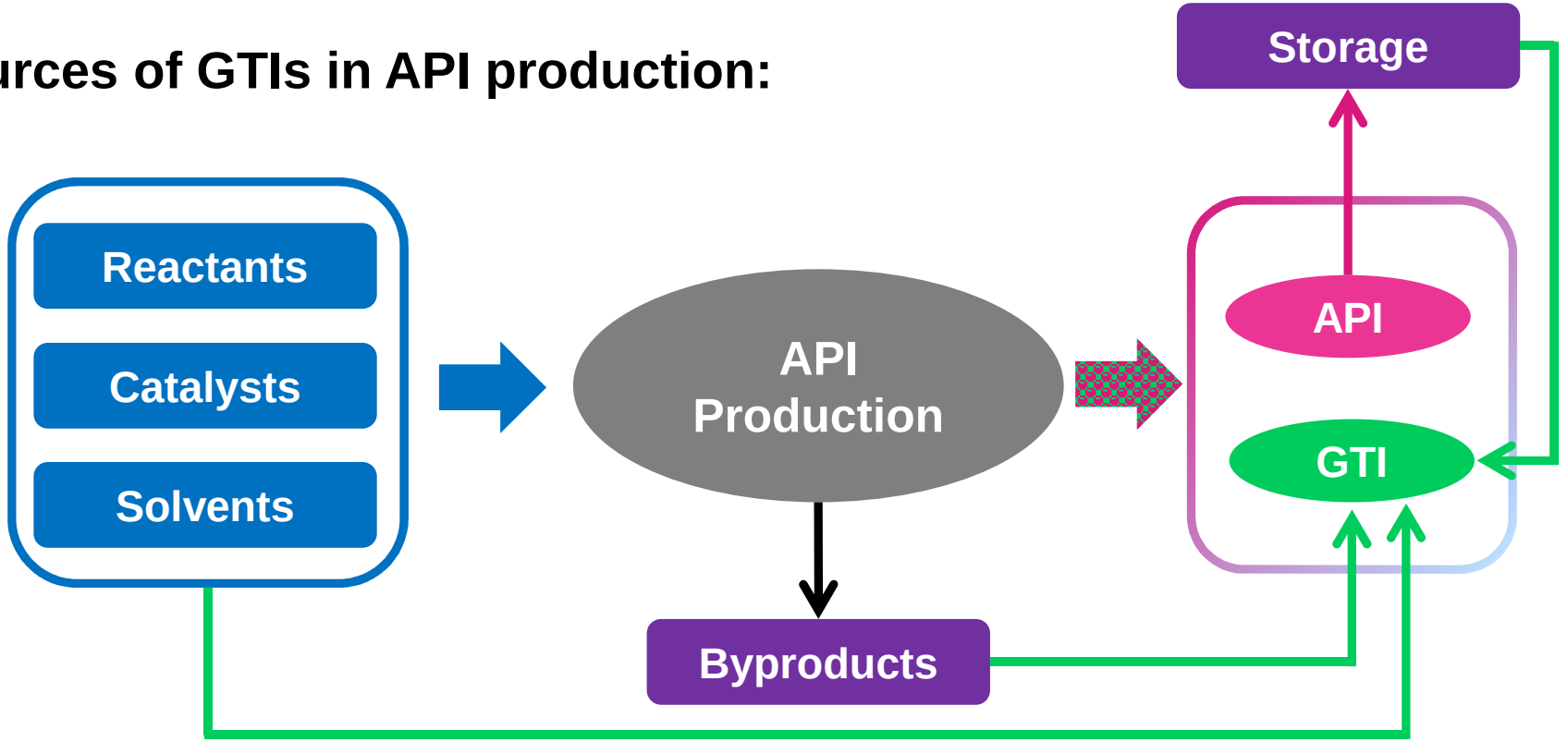
1. Introduction

❑ **Sources of GTIs in API production:**



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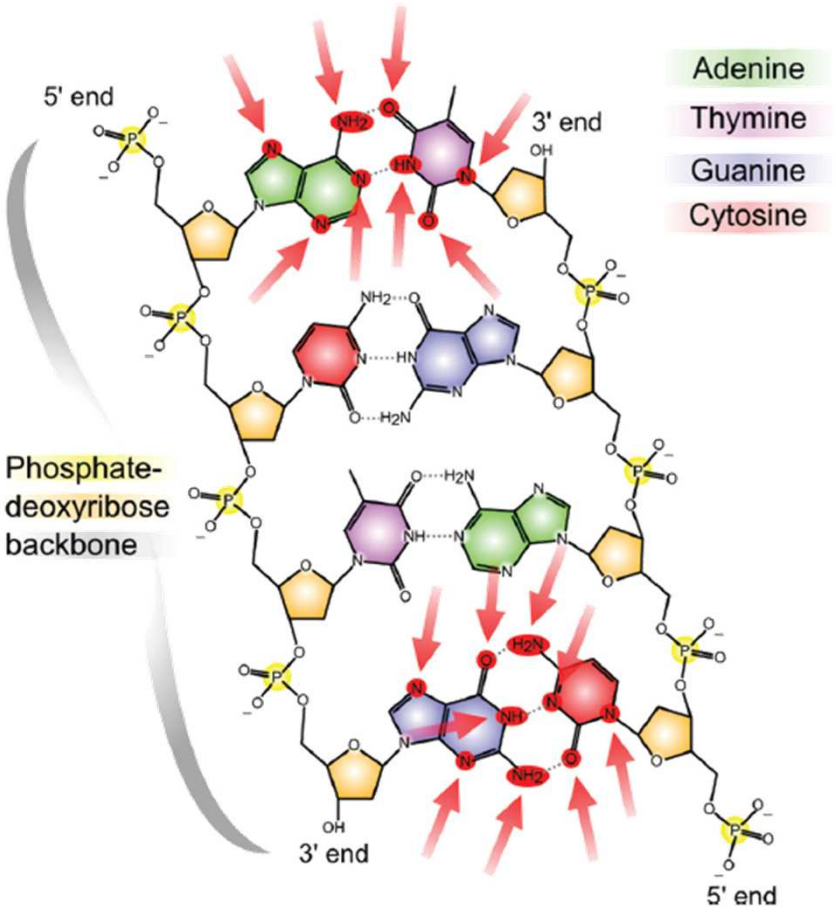
1. Introduction

- **GTIs:**
  - Broad range of chemical families (structural alerts)
  - Electrophilic species
  - React with DNA; can lead to strand breaks
  - Associated carcinogenic risk

→ Targeted nucleophilic sites of the DNA bases.



Székely G. et al., Chem. Rev., 2015, 115, 8182-8229.
(Madeleine Price Ball's Figure, GNU Free Documentation License)

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1. Introduction

❑ Removal of GTIs from APIs is of major importance:

- Strict regulations (FDA, EMEA) defined a **Threshold of Toxicological Concern** (**TTC**) :

TTC = 1.5 µg/day

- **TTC** corresponds to the probability of one patient in 1,000,000 to manifest the risk of having cancer.
- Below the **TTC** there is no appreciable risk to human health.
- GTI limits in APIs are calculated by dividing the **TTC** value by the maximum daily dose (g/day).

Székely G. et al., Sep. Purif. Technol., 2012, 86, 190-198.
EMEA Guidelines on the "Limits on Genotoxic Impurities", EMEA/CHMP/QWP/251344/2006, 2006.
FDA Guidance for Industry Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches; U.S. Department of Health and Human Services, 2008.

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1. Introduction

- **Several purification stages to remove GTIs from APIs**
- **Conventional techniques**

- Preparative column chromatography
- Recrystallization
- Phase exchange
- Resins
- Distillation…

Undesirable API losses

- To achieve **GTI** content in the API at **low levels** is in many cases **extremely difficult**.

- Production of APIs with low GTI contents is a **major** concern for API-manufacturing companies.

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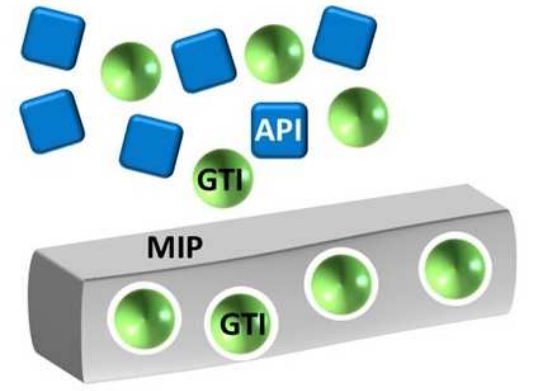
2. Main Goal

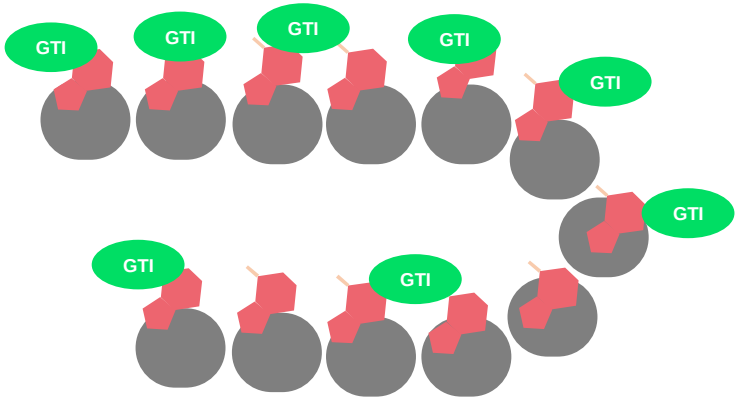
To design new adsorbers as GTI scavengers.

Advanced purification technique - organic solvent compatible platforms.

❑ **MIP (Molecularly Imprinted Polymer)**



❑ **DNA base containing polymer**



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3. Results and Discussion

3.1. MIP

- Concept
- Model compounds
- Synthesis
- Adsorber selection
- Experimental Design - GTI Binding
- Scale-up (SPE)
- Process Design

3.2. PBI-adenine

- Concept
- Model compounds
- Synthesis
- Adsorber selection
- Characterization
- Adsorber versatility
- Process Design

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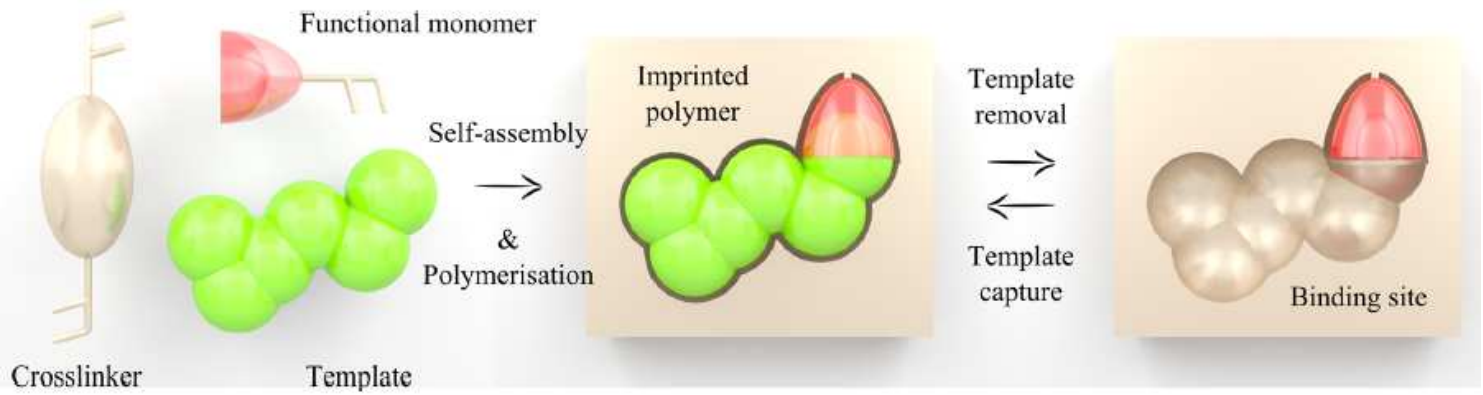
3.1. MIP: Concept

❑ Molecularly Imprinted Polymer (MIP)

- polymerization in the presence of a **template** molecule
- after polymerization, the template is removed and a **cavity** remains
- the polymer binds **specifically** target analytes, providing an accurate mechanism of **recognition**

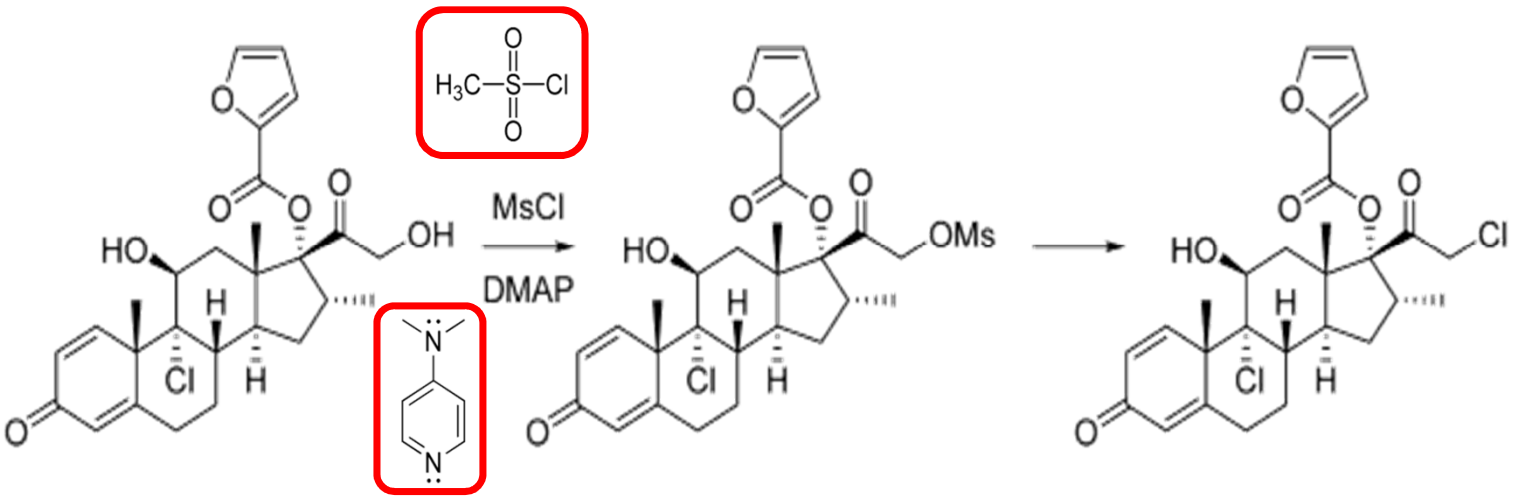
- Drug delivery systems
- Sensors
- Solid phase extraction
- Chromatography



Sellergren B. et al., WO2012172075 A1, 2012.
Székely G. et al., Sep.Purif. Technol., 2012, 86, 190-198.
Kupai et al., ACS Appl. Mater. Interfaces, 2015, 7, 9516-9525.

3.1. MIP: Model compounds

GTI: Methanesulfonyl chloride
(MsCl, 114.54 Da)



GTI: 4-Dimethylaminopyridine
(DMAP, 122.17 Da)

API: Mometasone furoate
(META, 521.43 Da)

API: glucocorticoid steroid used topically to reduce inflammation of the skin or in the airways:

- treatment of inflammatory skin disorders (such as eczema and psoriasis)
- allergic rhinitis (such as hay fever)
- asthma for patients unresponsive to less potent corticosteroids

Heggie W. et al., US 6177560, 2001.
Székely G. et al., Green Chem., 2013, 15, 210-225.

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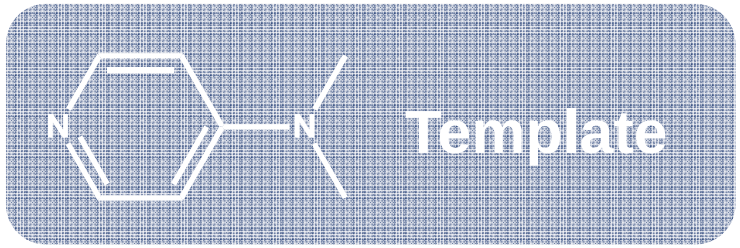
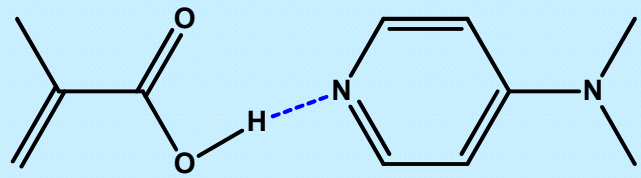
3.1. MIP: Synthesis

❑ DMAP (GTI) genotoxicity:



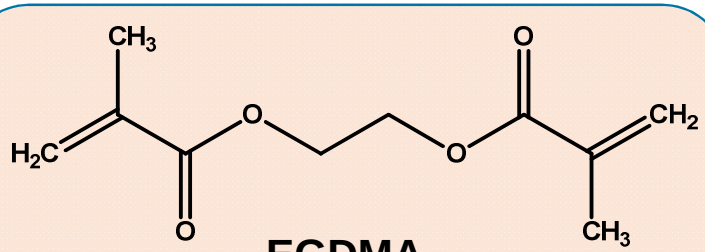


MAA
functional monomer
(methacrylic acid)

DMAP
template (T)

- two stuctural alerting groups: aromatic and alkyl amine
- aromatic amine *in vivo* decomposition leads to electrophilic reactive species:

- attack nucleophilic centre(s) of DNA
- associated carcinogenic risk



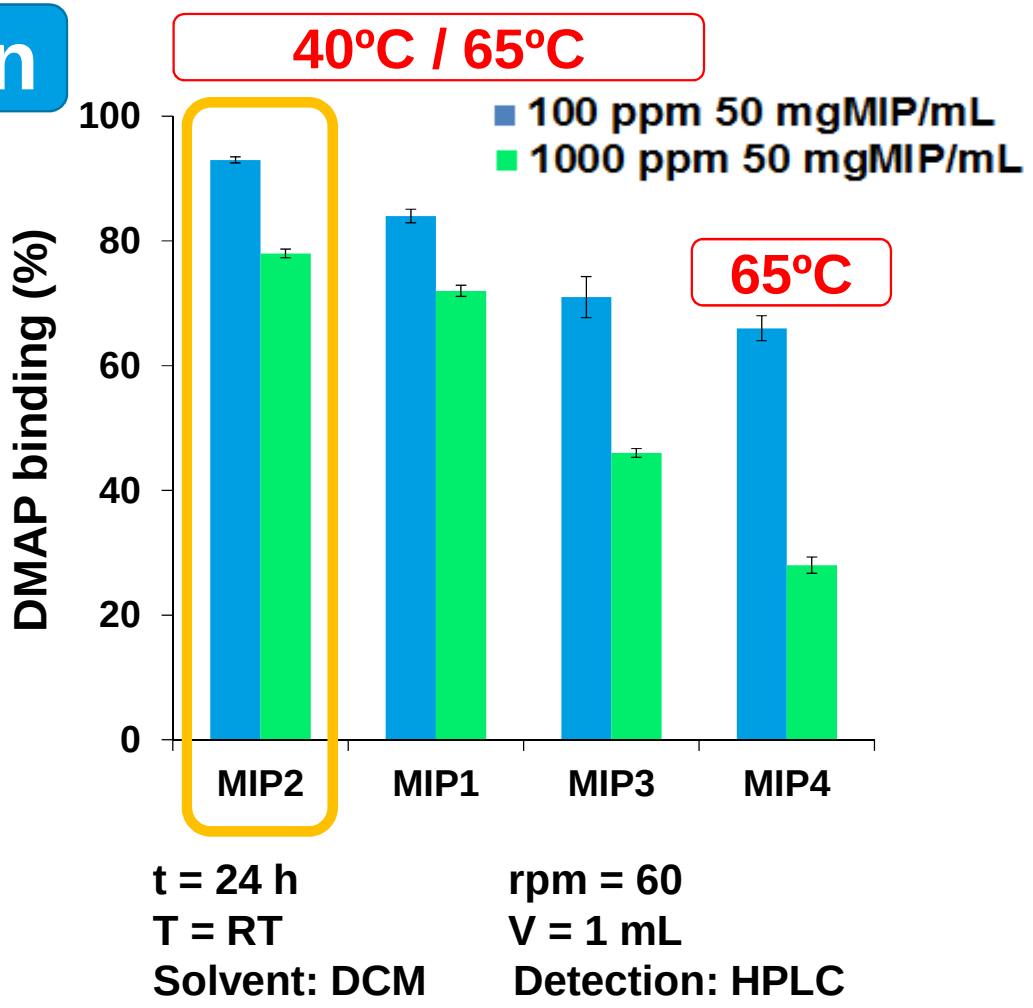
EGDMA
cross-linker
(ethylene glycol dimethacrylate)

Snodin et al., Org. Process Res. Dev., 2010, 14, 960-976.
Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

3.1. MIP: Adsorber Selection

- **Porogen:** DCM
- **T:** template
- **MAA:** functional monomer
- **EGDMA:** cross-linker
- **Method 1:** 16h at 40 ºC + 4 h at 65ºC
- **Method 2:** 16 h at 65ºC

	Stoichiometry (mmol)			
	T	**MAA**	**EGDMA**	**Method**
MIP1	0.1	0.4	1	1
MIP2	0.1	0.4	2	1
MIP3	0.4	0.4	4	1
MIP4	0.4	0.4	4	2



t = 24 h **rpm = 60**
T = RT **V = 1 mL**
Solvent: DCM **Detection: HPLC**

Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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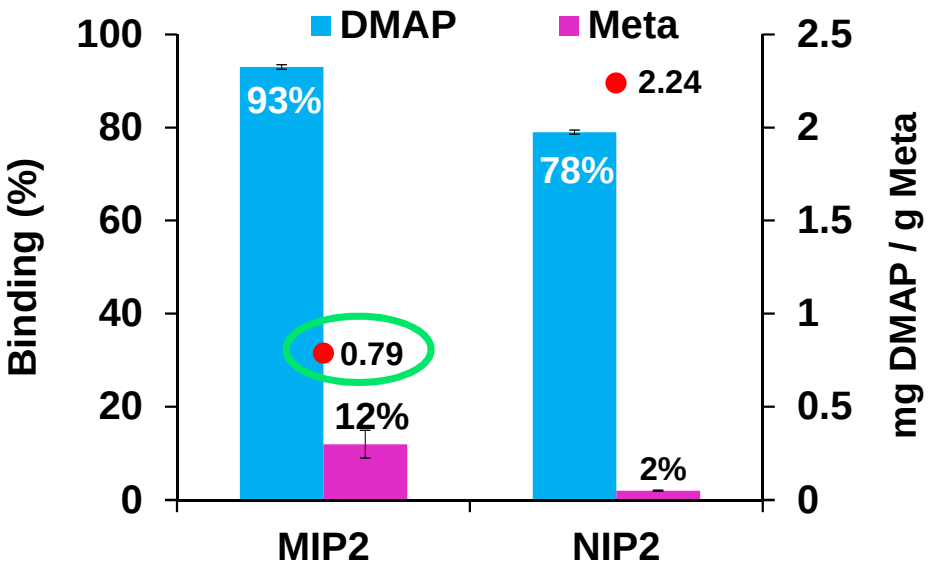
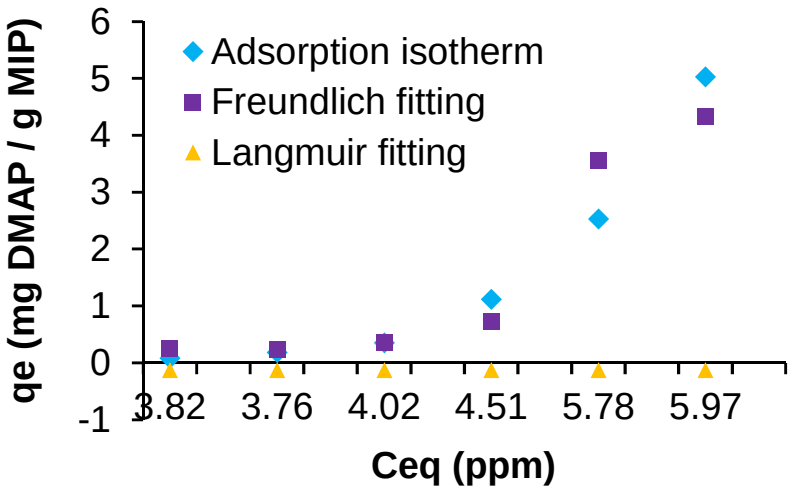
3.1. MIP: Adsorber selection



❑ Physical properties of **MIP2** and **NIP2** by multipoint BET method.

	Surface area ($m^2 \cdot g^{-1}$)	Pore volume ($cm^3 \cdot g^{-1}$)	Average pore Diameter (nm)
MIP2	**207**	0,024	5.6
NIP2	**242**	0,025	7.3

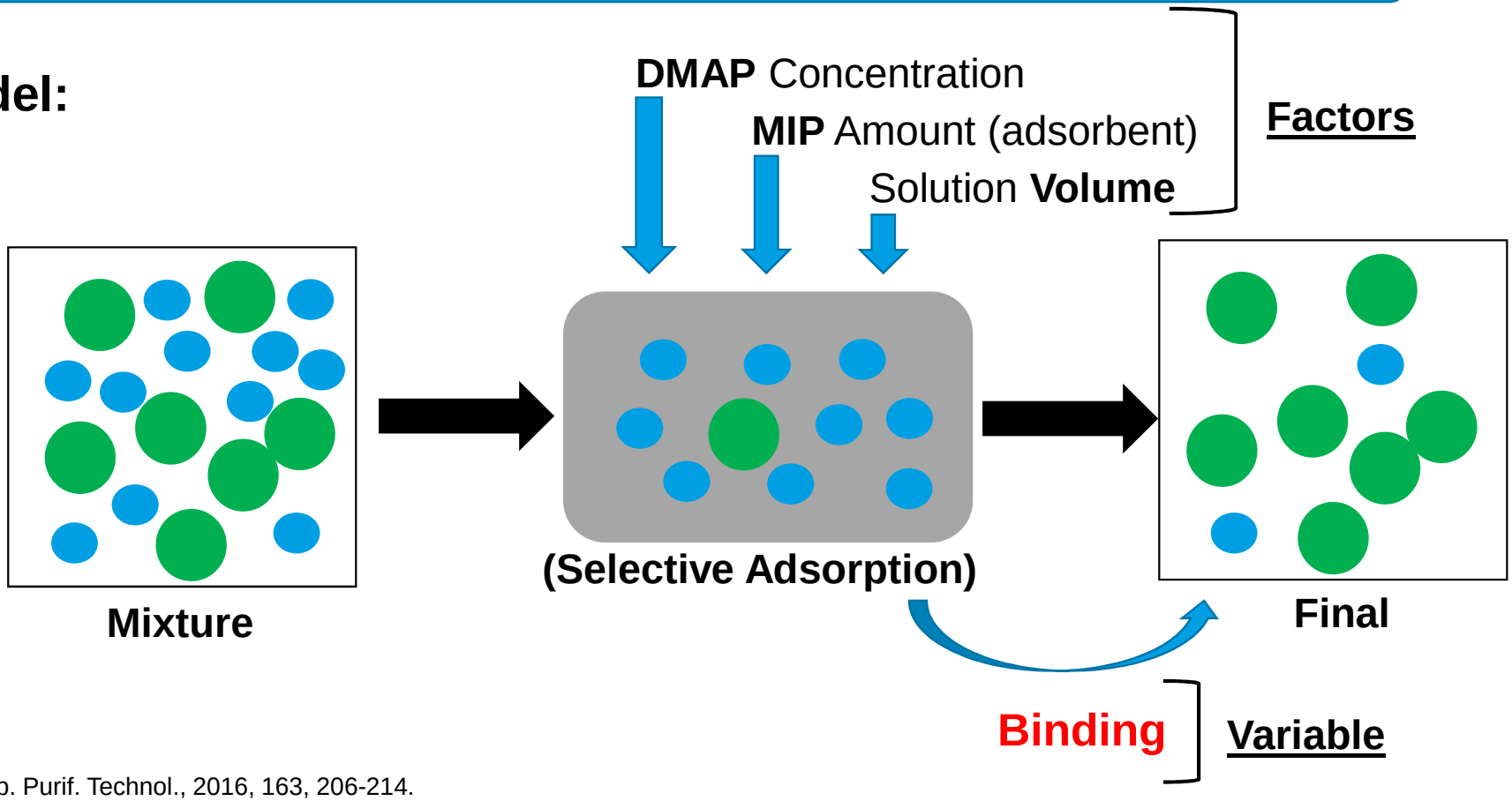


❑ Kinetic experiments: maximum 93% of GTI binding reached in less than 5 min.

Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

3.1. MIP: Experimental Design - GTI Binding

❑ **Model:**



Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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3.1. MIP: Experimental Design - GTI Binding

- Multifactorial design:

x_1 – DMAP concentration in ppm

x_2 – **MIP2** in mg

x_3 – Solution volume in mL

Factor	Low level (-1)	Central point (0)	High level (+1)
x_1 (ppm)	7	100	600
x_2 (mg)	37.5	75	100
x_3 (mL)	1.5	3	5

- Univariable design:

Bind. – binding percentage (DMAP removal from solution)

- A two level face-centered design was performed in order to optimize the 3 factors.

Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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3.1. MIP: Experimental Design - GTI Binding

	SS	DF	MS	F-value	p-value
DMAP	33.61	1	33.61	442.00	**0.0302**
DMAP²	3021.29				
MIP	830.7				
Volume	842.1				
DMAP x MIP	485.4				
DMAP x Volume	909.6				
Lack of fit	96.7				
Pure Error	0.07				
Total SS	6169.7				

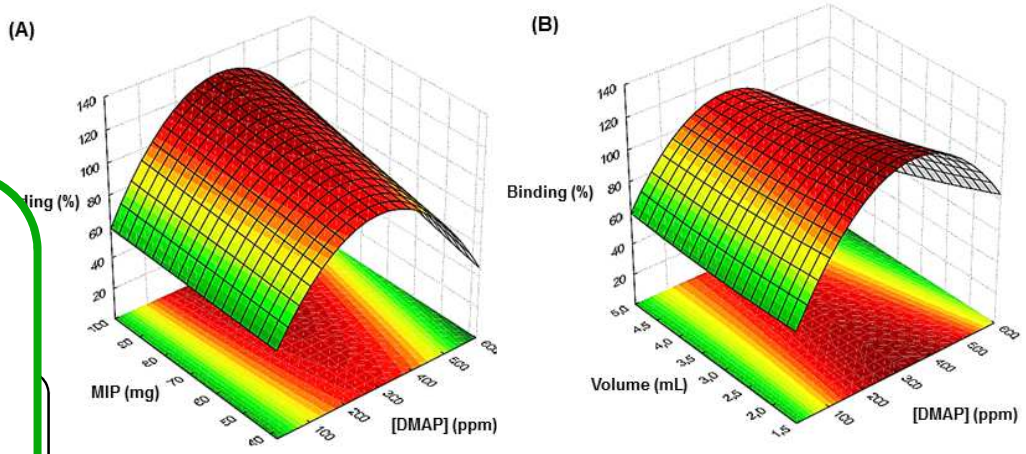
❑ **Model predictions:**

250 < **DMAP (ppm)** < 350

75 < **MIP (mg)** < 100

Volume = 1.5 mL

❑ ANOVA performed to the model with only statistically significant terms considered ($p < 0.05$).



❑ Response surface plots. Effect of:

A) DMAP concentration and MIP quantity;

B) DMAP concentration and solution volume on the binding.

Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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3.1. MIP: Scale-up (SPE)

	Batch	SPE 1 pass through
DMAP binding	93%	88%
Meta binding	12%	4%
mg DMAP / g Meta	**0.79**	**1.25**

T = RT

MIP2 = 50 mg

Detection: RP-HPLC

Solvent: DCM

flow: 0.15 – 0.42 mL/min

DMAP: 100 ppm

Meta: 10,000 ppm

Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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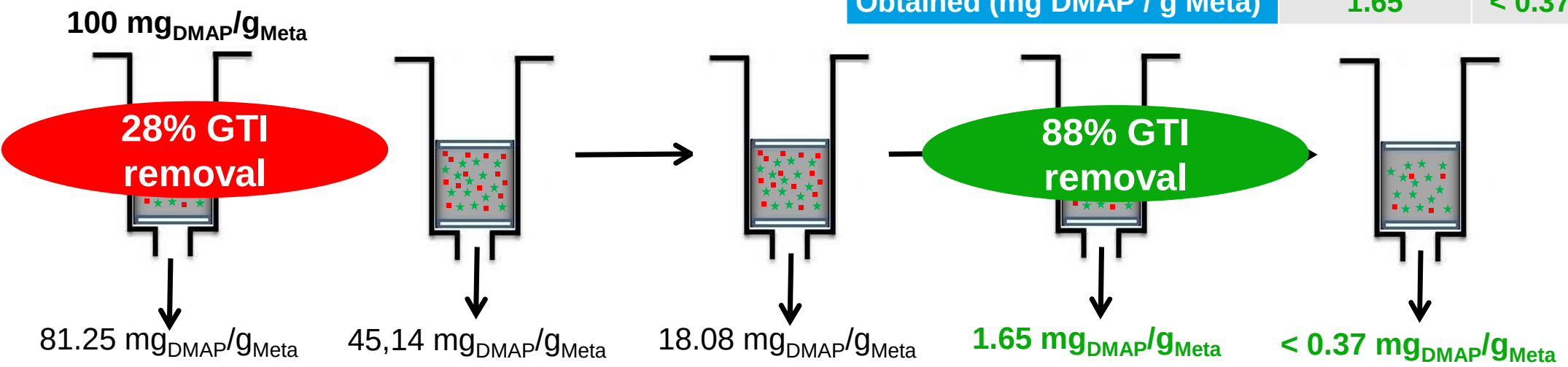
3.1. MIP: Process Design

❑ **SPE:**

Application	Nasal Spray	Cream
Maximum daily dose	200 µg	2 mg
Nº Steps	4	5
API loss	**16%**	**20%**
GTI removal	98%	> 99%
Target (mg DMAP / g Meta)	**7.50**	**0.75**
Obtained (mg DMAP / g Meta)	**1.65**	**< 0.37**



Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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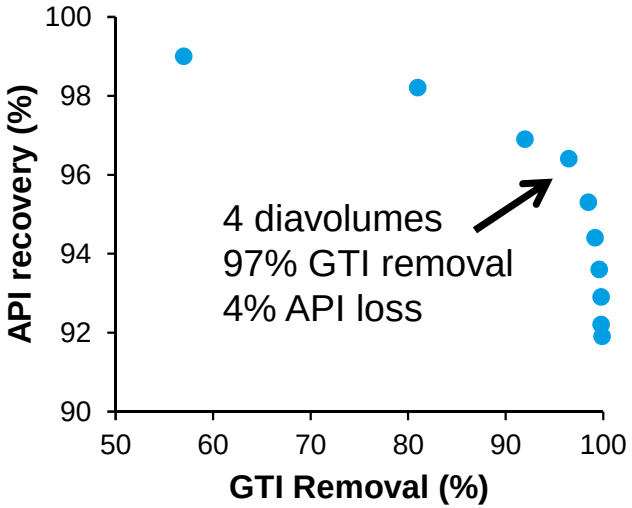
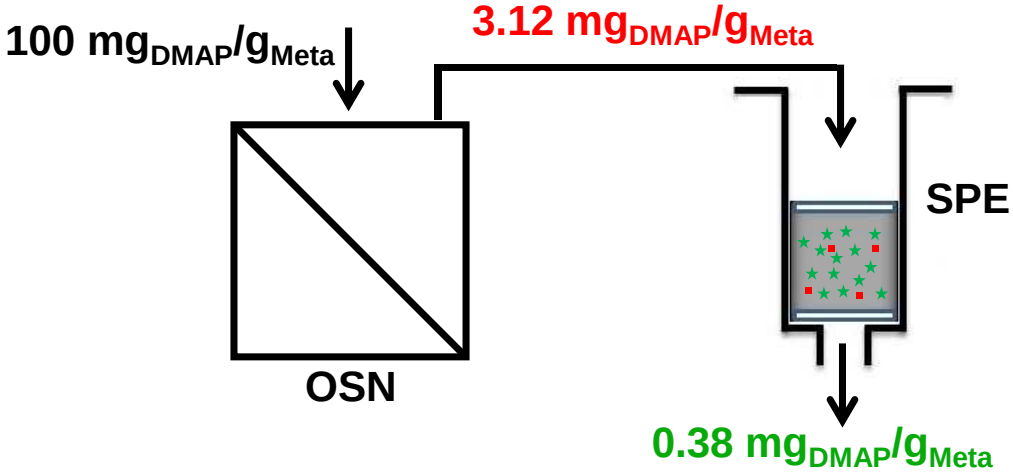
3.1. MIP: Process Design



	Rejection (%) at 10 bar
Meta (API)	99.0 ± 0.1
DMAP (GTI)	15.1 ± 0.3

❑**Hybrid process:**



Application	Nasal Spray	Cream
Nº Steps	1	2
API loss	**4%**	**8%**
Target (mg DMAP / g Meta)	7.50	0.75
Obtained (mg DMAP / g Meta)	3.12	0.38

Székely G. et al., Green Chem., 2013, 15, 210-225.
Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

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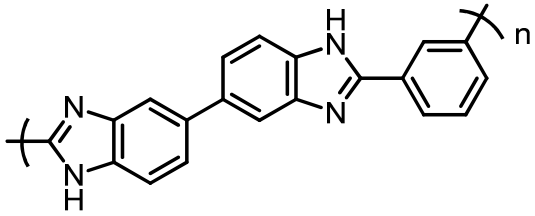
3.1. MIP: Process Design

Application	Nasal Spray		Cream	
Maximum daily dose	200 µg		2 mg	
Method	SPE	OSN	SPE	OSN + SPE
Nº Steps	4	1	5	2
API loss	16%	4%	20%	8%
GTI removal (%)	98%	97%	> 99%	> 99%
Target (mg DMAP / g Meta)	7.50		0.75	
Obtained (mg DMAP / g Meta)	1.65	3.12	< 0.37	0.38

Esteves T. et al., Sep. Purif. Technol., 2016, 163, 206-214.

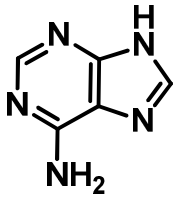
3.2. PBI-adenine: Concept

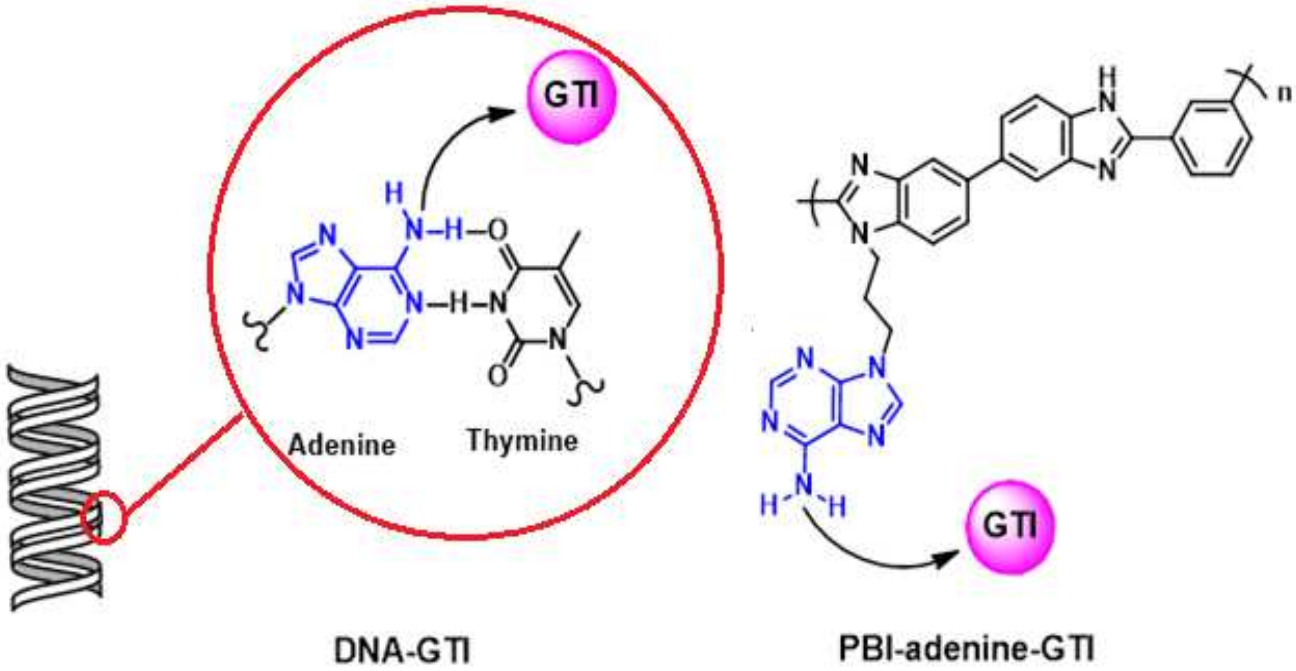
❑ **Polymer: PBI**



(polybenzimidazole)

❑ **DNA base: adenine**





Vicente A. I. et al., Sep. Purif. Technol., 2017, 179, 438-448.

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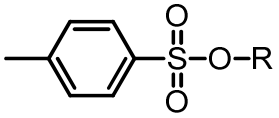
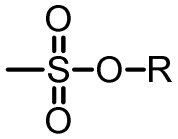
3.2. PBI-adenine: Model Compounds

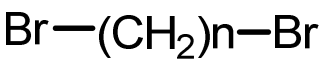
❑ GTIs: DNA alkylating agents



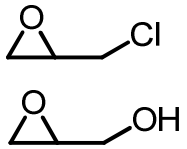
- **Alkyl tosylate**



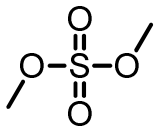
- **Alkyl mesylate**



- **Dihaloalkane**

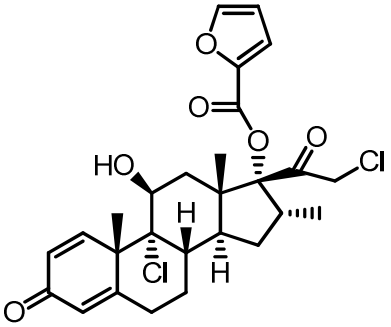


- **Epoxide**

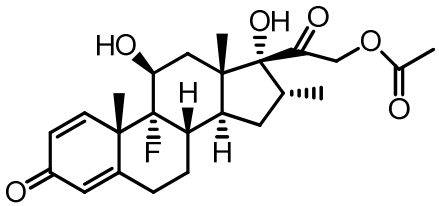


- **Dimethyl sulfate**

❑ APIs



- **Meta**



- **Beta**

Vicente A. I. et al., Sep. Purif. Technol., 2017, 179, 438-448.
Esteves T. et al., 2017, manuscript under preparation.

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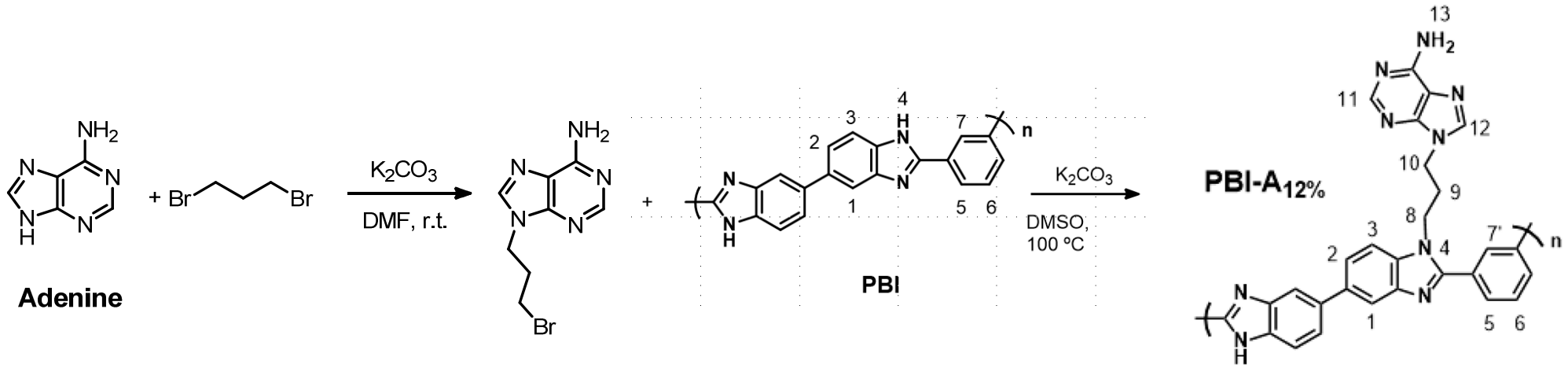
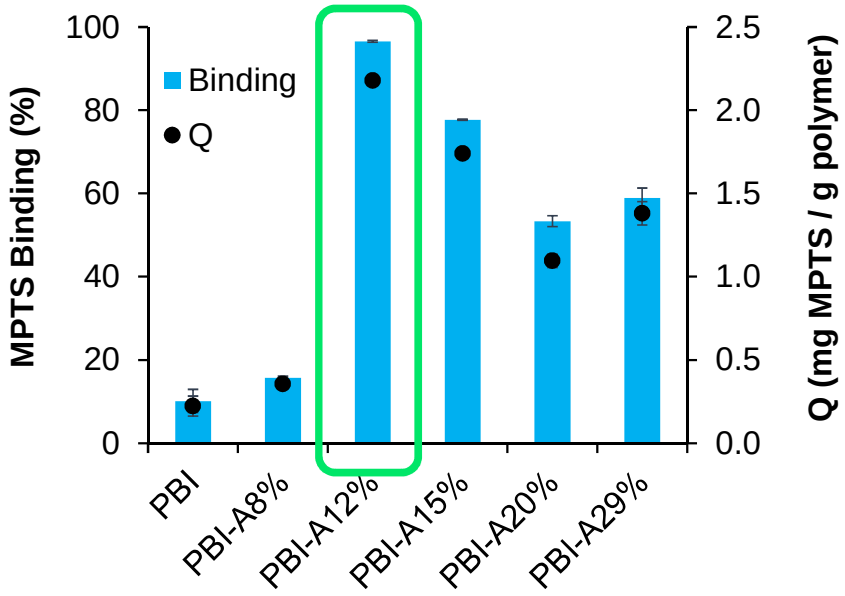
3.2. PBI-adenine: Synthesis

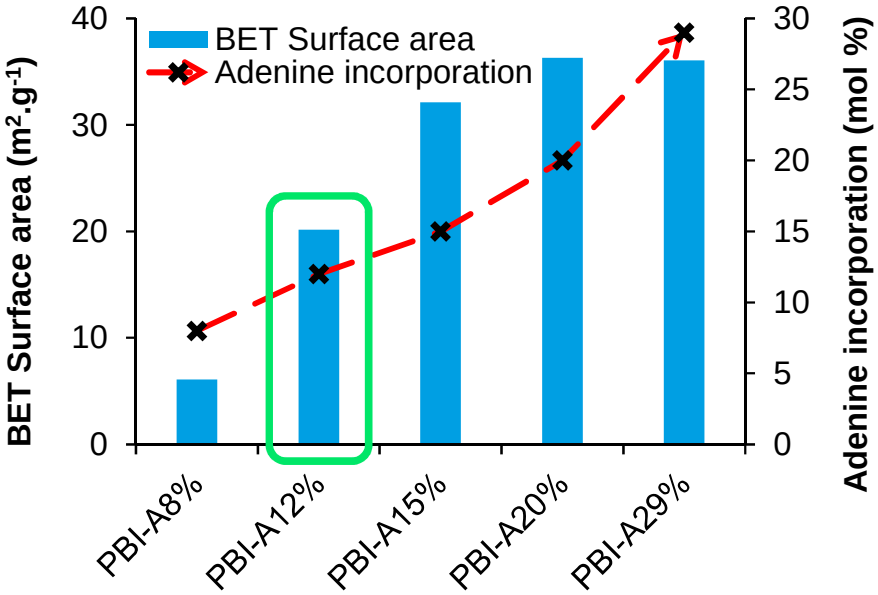
❑ **Synthetic strategy for alkylation of adenine and the PBI-$A_{x\%}$ polymer:**



Vicente A. I. et al., Sep. Purif. Technol., 2017, 179, 438-448.

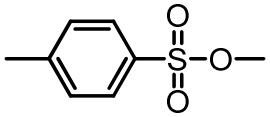
3.2. PBI-adenine: Adsorber selection





t = 24 h
T = RT
Solvent: DCM

rpm = 200
V = 1 mL
Detection: HPLC



MPTS

Vicente A. I. et al., Sep. Purif. Technol., 2017, 179, 438-448.

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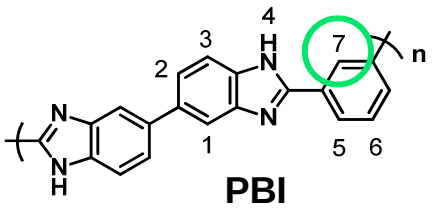
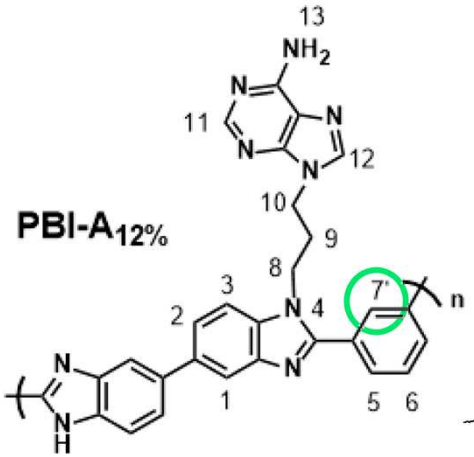
3.2. PBI-adenine: Characterization

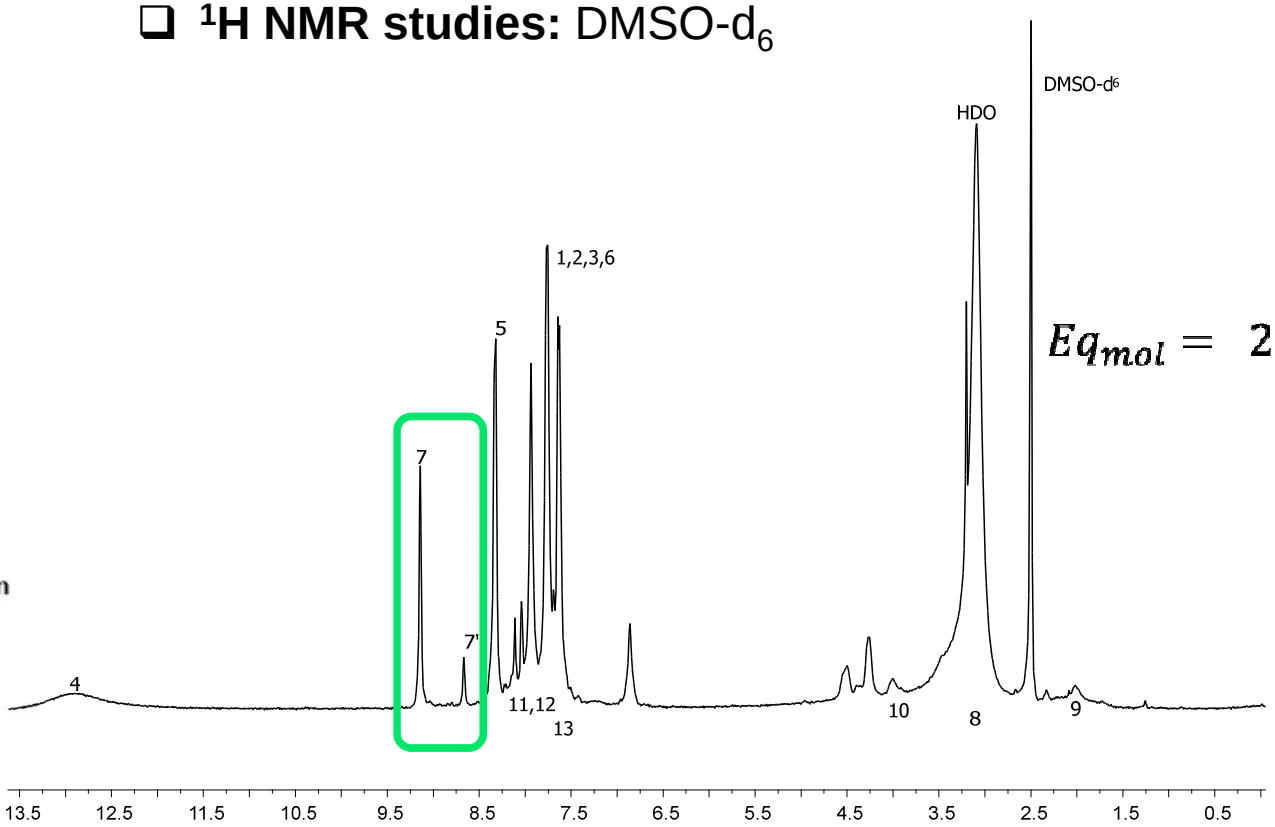


PBI



PBI-A$_{12\%}$

❑ **^{1}H NMR studies:** DMSO-d$_6$



$$Eq_{mol} = 2\frac{A_{8.7\ ppm}}{A_{8.7\ ppm} + A_{9.1\ ppm}}$$

Vicente A. I. et al., Sep. Purif. Technol., 2017, 179, 438-448.

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4. Concluding Remarks: MIP

- A **MIP** designed to target DMAP GTI was sucessfully synthesized.

- Efective removal of **93%** of DMAP (2 mg DMAP / g MIP) with a loss of **12%** of Meta (API) due to non-specific binding in batch experiments.

- Two step **hybrid process** design:

  - **OSN + SPE (MIP)**
  - **92% API recovery**
  - **> 99% GTI removal**

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4. Concluding Remarks: PBI-adenine

- An **adsorber** was successfully designed to target **DNA alkylating agents**.

- Process design:
  - Efective removal of **96%** of **MPTS** (2 mg MPTS / g PBI-$A_{12\%}$)

5. Acknowledgements

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