



A hybrid approach to reach stringent low genotoxic impurity contents in active pharmaceutical ingredients: Combining molecularly imprinted polymers and organic solvent nanofiltration for removal of 1,3-diisopropylurea

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ABSTRACT

The present study evaluates the adequacy of different approaches for the removal of 1,3-diisopropylurea (IPU), a potentially genotoxic impurity (GTI) from active pharmaceutical ingredients (APIs). The use of Organic solvent nanofiltration (OSN) to separate APIs and IPU, based on their molecular size difference was first evaluated using different membranes and solvents, and GMT-oNF-2 membrane was tested in diafiltration mode for the removal of IPU when dissolved in dichloromethane (DCM). A diafiltration dilution ratio of 3 was found as optimum to achieve 90% removal of IPU with a 2.5% loss of the model API. A novel IPU-selective Molecularly Imprinted Polymer (MIP) was then used for final polishing to remove remaining IPU. Hence, below 100 ppm IPU, IPU removal of 83% was achieved in one single stage. A selective elution system was also developed, consisting of the use of methyl isobutyl ketone (MIBK) in the first steps to recover virtually all the API that binds non-specifically to the MIP scavenger and methanol on the later steps to remove IPU and recover the MIP. The selectivity and stability of the MIP scavenger was validated over 18 independent operations using the same MIP sorbent. The combination of OSN with a diafiltration dilution ratio of 3 and a single MIP polishing stage allows reducing the IPU contamination from 100 mgIPU/gAPI to 2 mgIPU/gAPI with an API loss, mostly at the OSN stage, of about 3%. The results obtained using the hybrid OSN–MIP process were compared to those using MIP scavengers or OSN alone. The MIP–OSN hybrid approach, combining the advantages of both techniques, offers an attractive new approach for API purification.

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1. Introduction

Carbodiimides are often used as stabilizing, coupling and condensing agents in the preparation of peptides, nucleotides, various dehydration reagents in organic syntheses [1–3] and activators of carboxylic acids towards amide or ester formation [4]. However, the direct and unavoidable hydrolysis products of such carbodiimides are ureas, such as IPU, which becomes a GTI [5] in APIs. The feasibility of OSN has been successfully tested by the authors to remove GTIs from API post-reaction streams in diafiltration mode. In this mode fresh solvent was continuously added to the

OSN retentate in order to push the smaller GTIs through the membrane, whilst the larger APIs were retained [6]. The success and effectiveness of this separation, with high GTI removal and low API loss, depends on the difference of molecular size between the GTI and the API and on the corresponding membrane rejections.

IPU has a molecular weight of 144 g/mol and thus is partially retained by OSN membranes, thus to obtain significant IPU removal requires operation of the diafiltrations at higher dilution ratios. However, pushing more solvent through the membrane also leads to undesirable higher API losses, which is unacceptable in an industrial scenario. Moreover, membrane rejection for the solutes depends on various factors e.g. the membrane itself, solvent used, applied pressure, solute properties and so on. Considering rejections of about 25% and 99%, respectively for a GTI and API, one can calculate about 90% GTI removal (with an API loss of 3%) for a dilution ratio of 3. However, applying a dilution ratio of 5 would allow for a GTI removal of 97% at the cost of 5% API loss, which for some APIs, can have a significant economic negative impact.

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Therefore, to address the challenge on removing IPU from API post reaction solutions, we have reported on novel IPU imprinted polymers displaying strong affinity to IPU in presence of high levels of APIs. A detailed description of the preparation, characterization and recognition properties of these scavengers has been reported in an accompanying publication [7]. Two imprinted polymers, MIP1 and MIP2, and the two corresponding non-imprinted polymers, NIP1 and NIP2, were prepared in the absence (MIP1 and NIP1) and in the presence (MIP2 and NIP2) of 1,2,2,6,6-pentamethylpiperidine (PMP) base (Fig. 1). Since among these four hybrid process candidate MIPs the highest IPU binding was achieved with MIP2 [7] it was selected for the present study. The composition of the scavengers is shown in Table 1.

The use of the novel MIPs resulted in efficient removal of IPU in a single stage, about 80% IPU removal, even when in the presence of high API concentrations. However, under such conditions, a significant fraction of the API, about 15%, binds non-specifically to the MIP. Thus one of the aims in this study is to develop an improved MIP washing procedure that (i) allows quantitative recovery of the API which is non-specifically bound to the MIP in the first elution steps and (ii) removal of the IPU in the second elution steps, avoiding back contamination of API and thus allowing recycling of the MIP. A second aim is to evaluate strategies for high removals of IPU, achieving consistent low values of IPU contaminant, essentially through the combination of OSN and MIP in a hybrid process for purification of the API. Combination of OSN with other purification techniques can already be found in the literature [8–11] but to the best of our knowledge OSN–MIP hybrid processes have not been reported yet.

The previous equilibrium studies using MIP2 [7] shows that removal is limited to a maximum of about 80% of the total IPU. Therefore, high IPU removal, using MIP2 alone, requires a multistage procedure, in which the number of steps will depend on the initial and target threshold IPU concentration. Notice, also that the preliminary results giving 80% IPU removal were obtained with loads of MIP2 at 50 g/l in DCM at ratios of 2 mgIPU/gMIP2. The kinetic data indicate that about 22 h is required to reach equilibrium in this case. Therefore, instead of using a multistage procedure based in MIP2 alone and targeting directly the API post reaction stream, the current study evaluates the use of OSN followed by a MIP stage as depicted in Fig. 2. In this approach,

Table 1
Composition of IPU selective scavengers.

	Composition	Stoichiometry	Method
MIP1	IPU/MAA/EDMA	0.1/0.4/2	ABDV 40–60 °C
MIP2	IPU/PMP/MAA/EDMA	0.1/0.1/0.4/2	
NIP1	MAA/EDMA	0.4/2	
NIP2	PMP/MAA/EDMA	0.1/0.4/2	

the larger fraction of IPU is removed in the OSN permeate using a low dilution ratio. Thereafter, MIP2 is used to remove the IPU at lower concentrations from the retentate solution thus minimizing the load required of the relatively expensive MIP2 and number of MIP extraction steps.

2. Methods and materials

2.1. Materials

SolSep NF010206, GMT-oNF-2, MPF-44 were purchased as flat sheet nanofiltration membranes from SolSep BV (Netherlands), Borsig Membrane Technology GmbH (Germany) and Koch Membrane Systems Inc. (USA) respectively. The main features of the membranes reported by the supplier are summarized in Table 2, and additional information and imaging of the membranes can be found elsewhere [6]. Methanol (MeOH), and methyl ethyl ketone (MEK) was purchased from Merck (USA). Hydrochloric acid (HCl) and tetrahydrofuran (THF) were purchased from Fluka (Switzerland). Methyl isobutyl ketone (MIBK), dichloromethane (DCM) and 1,2,2,6,6-pentamethylpiperidine (PMP) were purchased from Sigma Aldrich (Germany). The protocol carried out for MIPs preparation is described by the authors in a previous paper [7]. Mometasone furoate (Meta) and 1,3-diisopropylurea (IPU) were kindly provided by Hovione FarmaCiencia SA (Portugal). The chemicals were used as received. All chemicals were of reagent grade or higher.

2.2. Apparatus

LCMS measurements were carried out on High Performance Liquid Chromatograph (HPLC) Waters model Alliance 2695

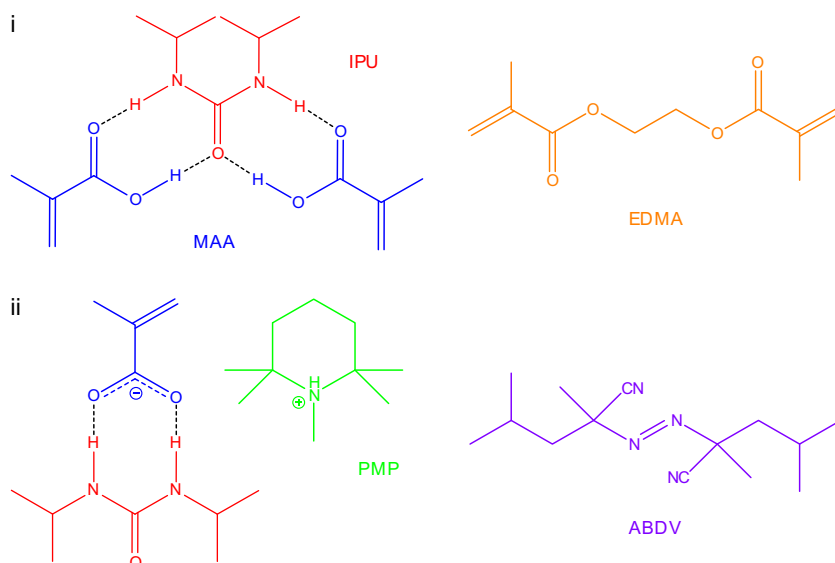


Fig. 1. Predicted host–guest interactions between the functional monomer methacrylic acid (MAA) and the template 1,3-diisopropylurea (IPU); (i) in the absence of 1,2,2,6,6-pentamethylpiperidine (PMP) base and (ii) in the presence of PMP base. Ethylenedimethacrylate (EDMA) and 2,2'-azobis(2,4-dimethyl valeronitrile) (ABDV) were used as crosslinker and initiator respectively.

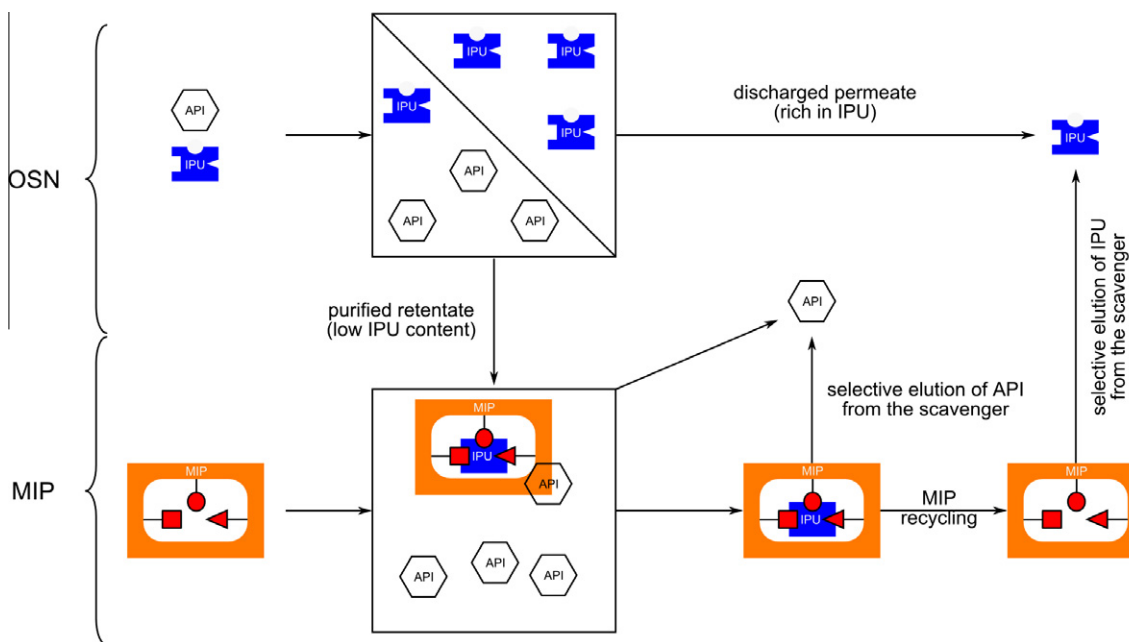


Fig. 2. Schematic principles of using OSN (stage 1) and MIP (stage 2) to remove GTI from API solutions.

Table 2

Description of GMT-oNF-2 and SolSep NF010206 membranes.

	GMT-oNF-2	SolSep NF010206	MPF-44
T_{\max} (°C)	80	120	40
p_{\max} (bar)	35	20	35
Separation characteristics	$R(93\%) \sim 327$ Da	$R(95\%) \sim 300$ Da	$R(90\%) \sim 250$ Da
Solvents	Alkanes, aromatics, alcohols, ethers and ketones	Alcohols and esters	Alkanes, aromatics, alcohols, ethers, ketones and haloalkanes
Type	Silicone polymer-based composite type	Not available	Proprietary composite type

equipped with Phenomenex, Kinetex 2.6 μm C18 100A, 100 \times 2.10 mm; Triple Quadrupole Mass Spectrometer for Liquid Chromatography Micromass model Quattro Micro equipped with an electrospray source; MassLynx Software V4.1 acquisition software. Solid phase extraction experiments were performed on Visiprep DL Vacuum Manifold equipped with 3 ml cartridges from Supelco (Germany). The METcell Cross-Flow System was purchased from Membrane Extraction Technology Ltd, now Evonik MET. METcell cross-flow system is a stainless steel, high-pressure stirred cell suitable for reverse osmosis (RO) and nanofiltration (NF) separations using aqueous and non-aqueous solvents. The system consists of high-pressure filtration cells, an organic solvent compatible gear pump and a tank base. The appropriate pressure was assured by use of pressured nitrogen from a cylinder fitted with a fine tuning reducer. An HPLC pump is connected to the tank during diafiltration in order to keep the volume of filtration constant.

2.3. Filtration procedure

The feed solutions were prepared at concentrations of 10,000 ppm for Meta and 1000 ppm for IPU in DCM. The membranes were preconditioned by filtering 300 ml of pure DCM at 20 bar through each membrane piece (52.8 cm^2) until solvent flux was constant. Rejections (R_x) for single solute solutions were estimated according to Eq. (1), for filtrations carried out at 10 and 20 bar. Feed volumes of 500 ml were used and bulk concentrations were measured in feed ($C_{F,x}$) and in the first 50 ml of permeate ($C_{P,x}$). Duplicate assays were carried out. Mass balances were closed using final permeate and retentate concentrations. Solvent fluxes

(ϕ) are calculated from Eq. (2) as function of volume permeated (V_p), membrane area (A_m) and filtration time (t):

$$R_x(\%) = \left(1 - \frac{C_{P,x}}{C_{F,x}}\right) \cdot 100 \quad (1)$$

$$\phi = \frac{V_p}{A_m \cdot t} \quad (2)$$

For diafiltration operation mode, GMT membranes and operating pressures of 10 bar were used. Fresh DCM was continuously added to the pressurized retentate at a constant rate in order to compensate for the permeate volume leaving the system. In this way the retentate volume was kept constant at a value of 500 ml. The diafiltration was interrupted periodically, after permeation of each 500 ml, and retentate and permeate concentrations were determined. Mass balances were closed and API loss and GTI removal estimated. The ratio of volume added (V_{Add}) per the initial 500 ml fed (V_{Feed}), is designated as dilution ratio (Eq. (3)):

$$D = \frac{V_{\text{Add}}}{V_{\text{Feed}}} \quad (3)$$

2.4. Selective elution of analytes from the MIP by SPE

Solid phase extraction (SPE) experiments were performed on Visiprep DL Vacuum Manifold equipped with 3 ml cartridges from Supelco (Germany) that were packed with 50 mg of MIP2 polymer between Teflon frits. Disposable liners were used in order to eliminate the possibility of cross-contamination when processing a new extraction on the same port. The MIP scavengers were

preconditioned with 1 ml 0.1 M PMP base in DCM. The mixture was left to stand for 5 min and then the preconditioning solution drained and 1 ml solution of the compounds of interest loaded on the polymers. The cartridge was sealed and the mixture was occasionally stirred during the 24-h equilibration time. The compounds were selectively eluted from the polymers by selective washing and the eluants were collected in separate vials. The loading solutions and wash eluants were analyzed by LCMS. MIP samples were regenerated by washing with 0.1 M HCl in MeOH in a Soxhlet extractor overnight. To confirm that no IPU leaches from the MIP scavenger, 50 mg MIP2 was weighed in an HPLC vial and 1 ml DCM was added and the mixture was stirred for 24 h. The IPU content in the supernatant was determined by LCMS.

2.5. Swelling experiment

Swelling is measured by allowing a given volume of dry polymer, with a known particle size and weight, to equilibrate in a solvent. For comparison purposes not only the swelling of MIP2 but also of the corresponding IPU selective MIPs described in the literature [7] was estimated. 100 mg polymer with 25–50 μm diameter was placed in narrow vials, each was loaded with 2 ml of the following solvents: DCM, MIBK, MeOH and a solution of 0.1 M HCl in MeOH. The mixtures were stirred and left to stand overnight to reach equilibrium at which point the polymer does not absorb more solvent. The bulk volumes of the dry and swollen particles were calculated based on the height and diameter of the particle bed in the vial, and used to estimate the volume swelling ratio (VSR):

$$\text{VSR} = \frac{V_{\text{swollen particles}}}{V_{\text{dry particles}}} \quad (4)$$

Since the weight and the volume of the dry particles are known, an apparent density (d_{app}) can be calculated:

$$d_{\text{app}} = \frac{m_{\text{dry particles}}}{V_{\text{dry particles}}} \quad (5)$$

Specific swelling volume was calculated from the volume swelling ratio and apparent density:

$$V_{\text{swelling}} = \frac{\text{VSR}}{d_{\text{app}}} \quad (6)$$

3. Results and discussion

Meta and IPU, molecular weights 521 g/mol and 144 g/mol respectively, were selected, as model API and potential GTI for the current study. Combining the results of previous OSN studies [6] with the use of a novel MIP developed specifically for IPU, a novel process for effective removal of GTI from API was developed. First, the use of OSN is discussed with regard to membrane selection and the effect of using different solvents and applied pressures, as well as diafiltration dilution ratios. The development of an elution procedure is then described to recover the API bound to the MIP2 scavenger by non-specific interactions. The recovery of the MIP scavenger and the IPU washout efficiency was also evaluated. In the final section, the combination of OSN with MIP based purification is discussed.

3.1. Organic solvent nanofiltration evaluation

DCM is a solvent commonly used in industry and used for developing and testing of the novel MIP2 for IPU binding in the previous study. Therefore, DCM was used as the solvent in the preparation of model solutions used to evaluate separations using MIP, OSN and combinatory approaches. Most OSN membranes are

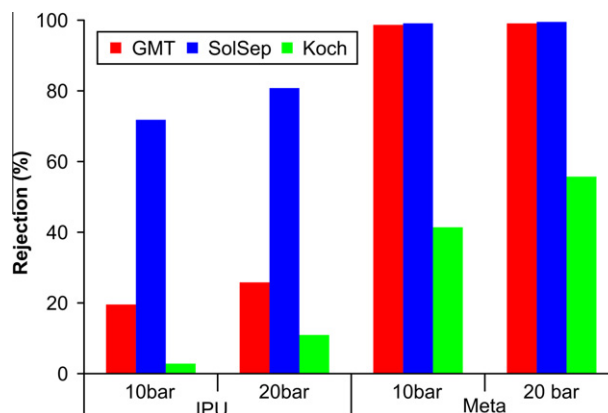


Fig. 3. Rejection of IPU and Meta at 10 bar and 20 bar in DCM on various OSN membranes.

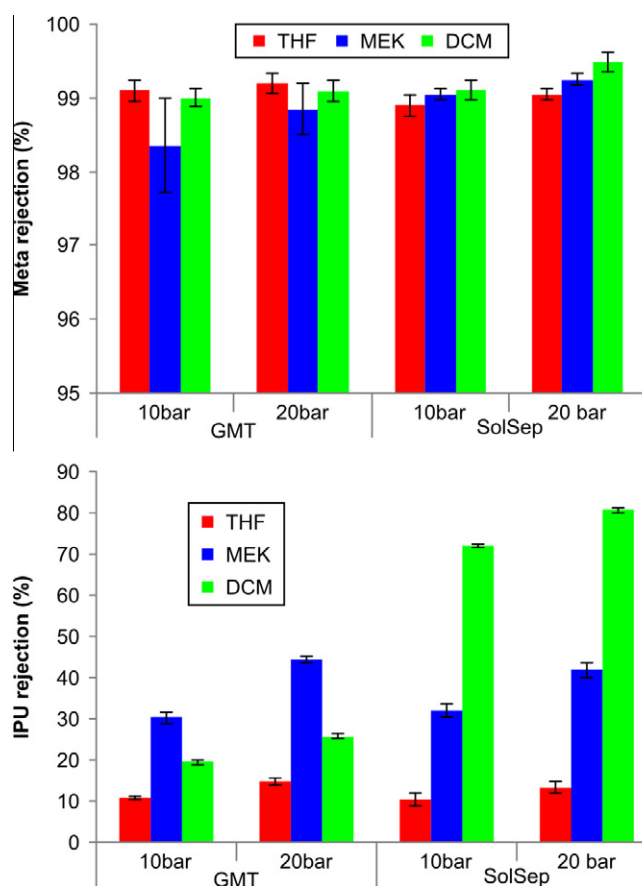


Fig. 4. Rejection comparison in DCM, THF and MEK solvents (top panel Meta, down panel IPU).

not compatible with DCM. Data reported previously show that membrane rejection of IPU and Meta was dependent on solvent and pressure [6]. Therefore, Fig. 3 compares the rejections obtained for IPU and Meta of the three thin film composite OSN membranes: SolSep NF010206, GMT-oNF-2 and MPF-44, tested in DCM at 10 bar and 20 bar in a cross-flow cell.

3.1.1. Membrane selection and solvent effect

The rejections obtained, show that when using DCM, not only the IPU (Mw 144 g/mol), but also a significant fraction of Meta

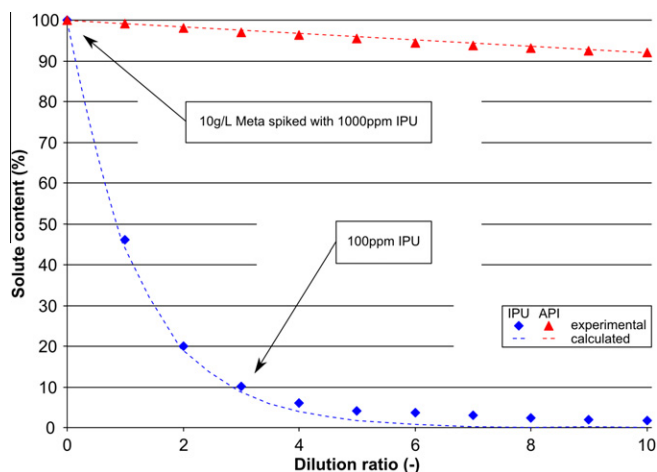


Fig. 5. OSN diafiltration: experimental results and calculated values.

(Mw 521 g/mol) crosses the MPF-44 membrane, indicating that in DCM the MWCO¹ should be significantly higher than the reported value of 250 Da [12] for this membrane or that the membrane is actually not entirely compatible with DCM. Thus, the use of MPF-44 for the intended separation was ruled out. On the other hand, IPU rejection by SolSep is significantly higher than expected according with previous studies [7] or the reported MWCO of 300 Da [13] for this membrane. Hence, in order to push a higher fraction of IPU into the permeate, a higher dilution ratio is needed, which also results in higher API loss. The results indicate that the GMT membrane is the most suitable for separating Meta from IPU when dissolved in DCM, with a lower IPU rejection and a high Meta rejection. The use of a lower applied pressure, at a value of 10 bar can also be exploited to increase GTI permeation.

The differences in rejections found for the same solute, but different solvents (Fig. 4), is most probably due to the extent of membrane swelling. In order to further explore the effect of solvents on the rejections of the tested solutes, the rejections of SolSep and GMT membranes obtained for IPU and Meta in DCM, THF and MEK were compared. The rejection of Meta is around 99% for the two membranes and three solvents tested. However, IPU rejections are greatly altered by the solvent system used. The polarity index for MEK, THF and DCM are 4.7, 4.0 and 3.5, respectively (with corresponding $\log P_{ow}$ of 0.26, 0.46 and 1.25). Both SolSep and GMT have lower rejections (10–15%) in THF than in MEK (30–45%), which could be explained by an increase of free volume as the more apolar solvent, THF, can cause slight swelling of the OSN membrane active layer. However, such correlation is not corroborated by the data for DCM (the most apolar solvent of the three), which drives rejections at intermediate values (20–25%) for GMT and significantly higher values (70–80%) for the SolSep membrane. It could be speculated that these results are due to DCM effects on transport mass transfer resistance in the active layers, the supporting layer of the membrane or interface between support and active layer. Such assessment requires studies that are outside of the scope of the present work. Even so the data collected is important for process development and membrane manufacture and the present work, confirms the use of GMT membrane for removal of IPU from Meta post reaction DCM stream by OSN diafiltration.

¹ Molecular weight cut off (MWCO) is the molecular weight of a solute that is expected to be 90% retained by a membrane, usually estimated experimentally by interpolation of calibrated solutes of the same chemical family.

3.1.2. Organic solvent nano diafiltration

Continuous addition of fresh DCM allows the operation of OSN in diafiltration mode and washing out of IPU from the system through the permeate whilst the API is retained by the membrane. Therefore, an experimental evaluation of this approach was performed. The mass balances closes out well – within a 3% error. Fig. 5 shows experimental results against the values calculated on the basis of mass balances and previously estimated IPU and Meta rejections for values of 19.8% and 99.0%, respectively. The detailed description of the calculation can be found in the authors' previous paper [6]. It is to be noted that the experimental results on IPU removal diverge from those calculated for lower IPU concentrations, which may indicate that rejection is concentration dependent and that IPU retention due to adsorption phenomena in the membrane becomes significant for lower IPU concentrations.

Overall, the results show that significant amount of IPU can be removed using the OSN diafiltration approach. However, such removal is more significant for the initial concentrations at the beginning of the cycle. As the IPU content decreases the efficiency of its removal also decreases. The rate of the API loss is sustained throughout the diafiltration, and achieves a value of 2.5% at a dilution rate of 3. At this dilution rate, a value of 100 ppm IPU content, corresponding to 90% removal, was achieved. Therefore, based on the diafiltration results, a dilution ratio 3 is judged to be a possible optimum end point for OSN diafiltration. Further treatment of the retentate by MIPs removes additional IPU.

3.2. The use of MIPs for GTI removal from APIs

MIP2 was developed using IPU as template as described previously and data indicate binding percentages for Meta and IPU of 15.1% and 82% to MIP2, respectively for a DCM solution containing 10,000 ppm Meta and 100 ppm IPU when loaded on MIP2 at 50 g/l [7]. To allow for a direct comparison of one single MIP2 step with OSN diafiltration, an additional experiment was performed with the MIP2 and Meta loadings as above, but with a higher IPU concentration of 1000 ppm. The results indicate that the percentage of IPU binding to the MIP2 was $49.9 \pm 0.4\%$, corresponding to about 495 ± 4 ppm of IPU equilibrium concentration in solution. In other words, the results show that (i) MIP2 is capable of effectively removing IPU at lower concentrations and in contrary (ii) the OSN diafiltration approach is superior to remove IPU at higher concentrations: IPU in solution is reduced from 1000 ppm to about 100 ppm (vs. about 500 ppm for MIP2 system) with only 2.5% loss of API (vs. 15.1% in the MIP2 system) at dilution ratio of 3.

MIP systems could be explored for further removal of IPU using a cascade type methodology where the supernatant of the first step is submitted to additional steps for sequential removal of further IPU. However, if this approach were to be applied other issues such as minimization of API loss and regeneration of MIP2 would have to be addressed. Since a significant amount of API ends up bound to the MIP in the first step, a proper recovery step for such bound API – without back contamination of the IPU – would be crucial to improve the competitiveness of the MIP application. The recovery of spent MIP2 is also an important consideration from a cost efficiency perspective. A single solvent or a mixed solvent system, able to wash off only the API in the first elution steps and then the specifically bound IPU in the following elution steps, would resolve this problem. The screening for such solvent systems is discussed in the following section.

3.2.1. API recovery and MIP regeneration: selection of eluent

As discussed before, the MIP selectively binds IPU but also binds about 12–15% of the API in each step through non-specific interactions, which represents unacceptable loss of product. Several solvents were screened for their ability to dissolve API (Meta), but

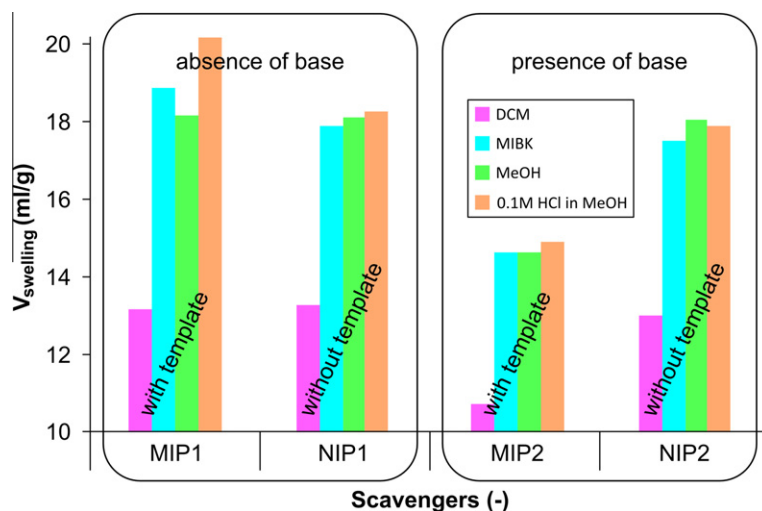


Fig. 6. Specific swelling volumes of the IPU selective MIPs.

not the potential GTI (IPU), by exploring the different strengths of interactions between polymer with GTI and API in these solvents. The objective was to be able to remove easily the API in an initial elution step and significant IPU removal in additional elution steps. Polymer swelling was also tested in the solvents used and these swelled polymers employed in binding tests or solid phase extractions. Fig. 6 shows the specific swelling volume estimated for MIP2 (the polymer selected for the development of the hybrid process) and MIP1, as well as the respective non imprinted polymers NIP1 and NIP2 from the literature [7]. Interestingly, the lowest swellings were observed in DCM, the solvent used for preparation and IPU removal tests, than for any of the other solvents. In particular, MIP2 which has the highest affinity towards IPU has the lowest swelling. Swelling leads to significant changes in the properties of the polymers, affecting accessibility of the binding sites, and thus, the adsorption–desorption process. Therefore, removal of IPU in a solvent with low swelling properties, such as DCM, and desorption in solvents, such as MIBK and MeOH that swell the polymer significantly can actually be beneficial, since it promotes easier removal from the enlarged polymer pores [14].

DCM is the solvent in which the initial binding step takes place. Therefore, the use of DCM for further API elution would be ideal, avoiding solvent swap and allowing recovery of the API fraction in the original solvent. However, the experimental data shown in Fig. 7 indicates that the use of additional pure DCM results in back contaminating of the Meta, since DCM partly elutes IPU as well. This is an expected result, given the high solubility of IPU in this solvent.

Therefore, in order to recover Meta from the MIP2 scavenger, a strategy of using two elution solvents was evaluated. Following preliminary results, MIBK was selected as the first solvent to be used. Meta is highly soluble, but IPU is insoluble in this solvent. Thus, the use of pure MIBK allows recovery of non-specifically bound Meta in the first or first two washes (Fig. 7b and c) without significant cross contamination with IPU. This strategy has the drawback of recovering API in a solvent different from DCM, thus implying an undesirable solvent swap for the next processing step. Due to the high solubility of IPU in DCM and MeOH, these solvents were selected as secondary elution solvents to wash out the IPU, regenerating the MIP2. Methanol is a high polarity solvent, capable of disrupting the H-bonds in the host–guest interaction therefore, a faster IPU removal is possible with MeOH than with DCM. 99% IPU removal was achieved in the washing performed with MIBK:MeOH (30:70). Therefore the MIBK/MeOH solvent system was selected to

regenerate the MIP2 scavenger. This demonstrates that an appropriate selection of solvents makes it possible to efficiently recover the fraction of 13–15% of API non-selectively bound to the MIP2 scavenger without contamination with IPU, addressing one of the main challenges identified in the use of MIPs.

3.2.2. Robustness of MIP2 recyclability

A second challenge is that, as IPU binding percentage decreases for higher IPU concentrations, removal of IPU down to the required low levels implies a sequence of several steps of IPU binding and MIP2 recycling, which is time consuming and cumbersome. Therefore, the multi-steps strategy often followed in OSN systems [15,16] is not so often applied in MIP systems.

Nevertheless, the novel MIP2 is particularly robust in terms of recyclability without loss of selectivity. Independently of operation mode, the option to recycle the MIP scavengers without any efficiency loss is crucial from an industrial point of view. The recyclability of the MIP2 scavenger is indicated by IPU binding values, which are between 80% and 85% for all the 18 assays performed sequentially using various polymer samples. These 18 assays were performed at 50 g/l of MIP2 in DCM and an IPU concentration of about 100 ppm. The results were assembled from several experiments reported in this article and in previous studies [7] which evaluated the binding of IPU either alone or in the presence of different APIs, through kinetic and thermodynamic studies with a variety of elution solvents. In all these experiments the final MIP2 regeneration employed was as follows: wash with 0.1 M HCl MeOH solution, dry in a vacuum oven and precondition with PMP base in DCM for further use.

3.3. Hybrid process for GTI detoxification: Exploring OSN diafiltration and MIP polishing of the retentate

Assessment of the OSN diafiltration and MIP2 systems independently was reported in the previous sections, showing that the OSN diafiltration is more suitable for higher IPU concentrations being able to reduce IPU levels from 1000 ppm to 100 ppm using a dilution ratio of 3 (with only 2.5% loss of Meta). However, IPU removal below 100 ppm by OSN was highly inefficient. On the other hand, based on the isotherms of IPU binding to MIP2 [7], the MIP approach suits the removal of IPU better from solutions where this solute is at low concentrations of about 100 ppm. A suitable solvent system was selected to recover API, non-selectively bound to the MIP2 scavenger. In this section a hybrid approach is

suggested and evaluated experimentally. Diafiltration is first used to remove the greater part of the IPU and MIP2 is then used to polish the retentate stream resulting in ultra low levels of IPU content in the final Meta solution.

The elution system developed in the previous section for the recovery of Meta and IPU was applied. A solution with 10,000 ppm Meta and 1000 ppm IPU in DCM was submitted to diafiltration using an OSN GMT membrane and an applied pressure of 10 bar. The OSN diafiltration operation, run at an average solvent flux of $50 \text{ L m}^{-2} \text{ h}^{-1}$ was stopped at a dilution ratio of 3. The retentate was fed to MIP2 at a load of 50 g/l in the same solvent, DCM, corresponding to ratios of 2 mgIPU/gMIP2. After 22 h, the MIP2 was collected from the purified Meta solution and MIP2 retained for further recovery of the non specific bound Meta. Pure MIBK was used in the two first washing steps, followed by MIBK/MeOH solutions with increasing MeOH content in washing steps 3–6. The results obtained are illustrated in Fig. 8 and follow expectations with virtually all of the Meta being recovered in the first two wash steps thus increasing

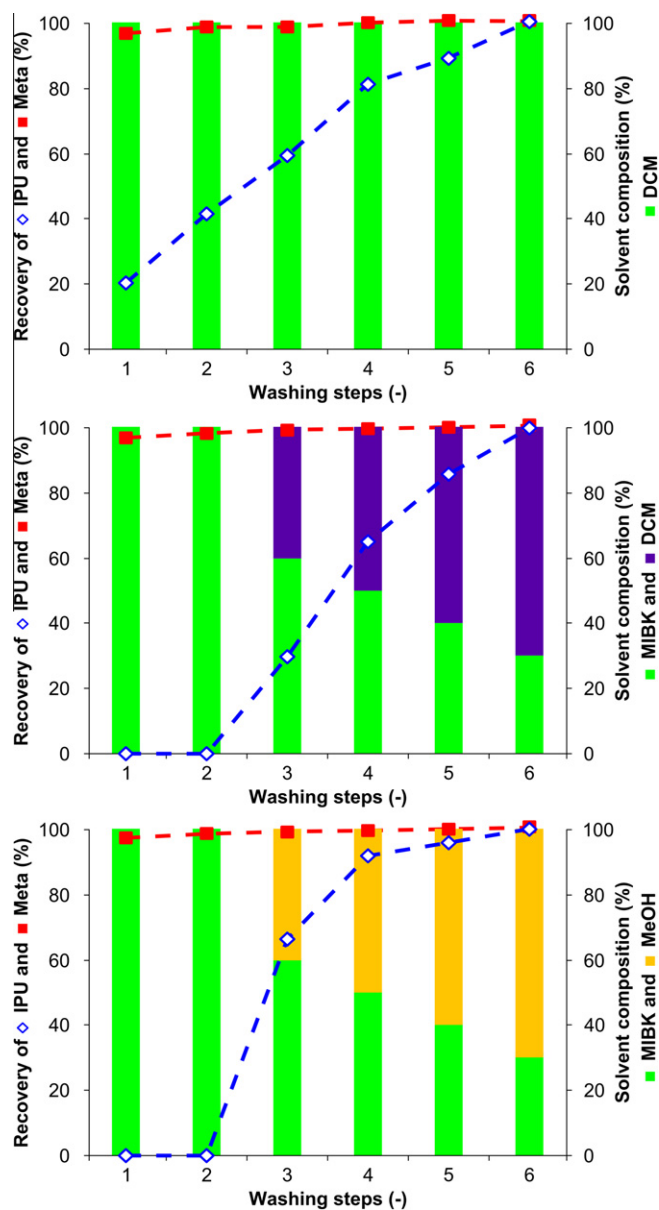


Fig. 7. Selective elution of Meta and IPU from MIP2: (i) elutant is DCM, (ii) first elutant is MIBK and then DCM content is increased, (iii) first elutant is MIBK and then the MeOH content is increased.

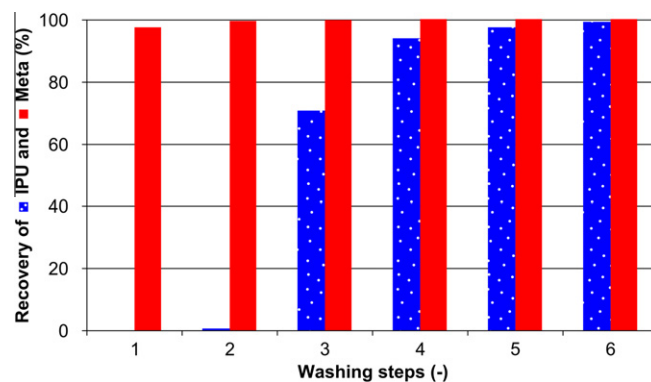


Fig. 8. Cumulative recovery of Meta and regeneration of the MIP2 scavenger after polishing the OSN retentate.

the recovery of Meta from 97.5% to 99.7%. The IPU was efficiently washed out of the MIP2 in the following steps.

Fig. 9 provides a detailed schematic diagram of the hybrid process including the quantification of Meta and IPU at the different phases. The bulk of the Meta, 97%, is retained by the membrane and 90% of the IPU is discharged in the permeate. At this point, there is still 10% of IPU that remains in the retentate which corresponds to 10 mgIPU/gMeta (1 wt.%). Therefore, the following MIP stage aims to decrease this level of contamination to the required levels.

The experimental data show that, using the appropriate systems of eluting solvents that it is possible, without significant additional Meta loss (97% yield of Meta), to bring down IPU levels to a value of 2 mgIPU/gMeta (2 ppm). Only one cycle using MIP was performed to obtain this level. Hence, assuming that in each cycle about 80% of the IPU is removed, by treatment of the supernatant I (Fig. 9) it should be possible to reduced IPU content by about an order of magnitude per MIP cycle. However, this additional purification is achieved at the cost of the generation of a MIBK stream (supernatant II in Fig. 9) in each cycle. The isolation of Meta therefore, involves a solvent swap back to the lower boiling point solvent, DCM. The removal of APIs from organic solvents by nanofiltration has been previously studied [17] and several groups have suggested the use of membranes for solvent exchanges [18–20] and for the recovery of solvents [21] in the pharmaceutical industry when heat sensitive compounds are involved or when the solvent has a high boiling point, making distillation unsuitable.

4. Conclusion

The studies reported in this paper show that OSN is an efficient process for removal of IPU from Meta at relatively high concentrations (100–1000 ppm) when using low dilution ratios to avoid excessive loss of API. For dilution ratios superior to 3, corresponding to IPU content in solution lower than 100 ppm, OSN becomes inefficient. On the other hand, the use of a novel MIP developed for the removal of IPU, MIP2, is more efficient when applied to IPU solutions in the lower concentrations range, with typical IPU removal efficiencies of 83%. Unfortunately, about 15–20% of Meta binds non-specifically to MIP2. To address this challenge an elution system was developed using MIBK to recover Meta in the first two steps and mixtures of MIBK/MeOH in the 4 subsequent steps to remove IPU from MIP2 thus recovering the MIP2 scavenger. The elution system allows for the removal of significant amounts of IPU from MIP2 with negligible Meta loss. However, the use of MIP alone for the purification of Meta would require a MIP cascade process which is time-consuming and would increase solvent usage.

Therefore, a hybrid process was developed which combines the advantages of molecular imprinting and organic solvent

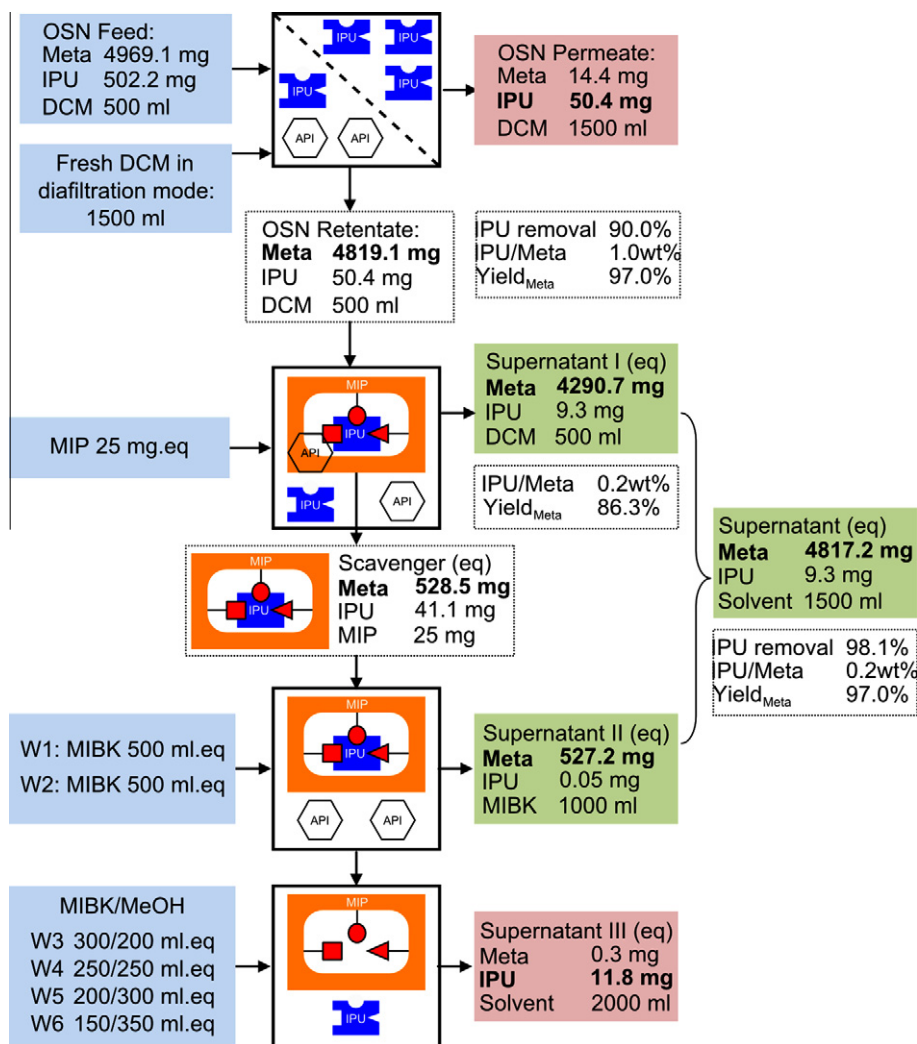


Fig. 9. Hybrid process – mass balance of the operation stages carried out.

nanofiltration. An initial OSN stage is applied at a low dilution ratio, where the diafiltration process is efficient and then the retentate containing a low amount of IPU is submitted to the novel imprinted polymer for further IPU removal. The transition between OSN and MIP stages takes place where the IPU concentration is approximately 100 ppm, a value particular suitable for MIP2 operations. Meta with significantly lower contamination of by IPU was achieved using this hybrid approach. MIP2 was also efficiently recovered and reutilized over 18 operations without loss of selectivity towards the target molecule, the potential GTI, IPU. This methodology can be used to explore other cases where ultra low concentrations of contaminants must be reached.

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