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Mendeleev Commun., 2020, **30**, 220–222

Mendeleev Communications

TiO_2-modified MALDI target for *in vitro* modeling of the oxidative biotransformation of diclofenac

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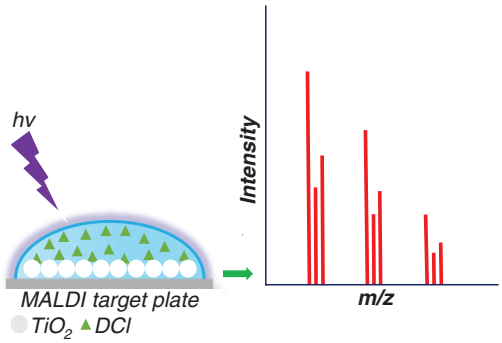
DOI: 10.1016/j.mencom.2020.03.030

The UV-induced photocatalytic oxidation in the presence of TiO_2 nanoparticles (UV/TiO_2-PCO) is a more adequate approach than electrochemical oxidation to simulate the oxidative metabolism of diclofenac based on the comparative analysis of oxidation products using high-resolution tandem mass spectrometry. A simple and fast high-throughput technique is proposed for modeling the oxidative metabolism, which involves UV/TiO_2-PCO performed directly on a MALDI target and subsequent analysis by matrix-assisted laser desorption/ionization mass spectrometry. The ranges and yields of diclofenac oxidation products obtained by the conventional bulk UV/TiO_2-PCO and the proposed on-target version are in excellent agreement.



Keywords: titanium dioxide, photocatalysis, photooxidation, drug metabolites, MALDI MS.

The metabolic conversion of xenobiotics into reactive products, commonly referred to as bioactivation, is a major determinant of their unpredicted biological activities including adverse side effects. An analysis of the oxidative metabolism of xenobiotics is important for drug development as it allows one to predict the potential toxicity of novel pharmacological entities.[1]

Since biotransformation analysis with the use of biological systems (liver microsomes, hepatocytic cell lines, and laboratory animals) is laborious and time-consuming, fast and simple methods for the nonenzymatic *in vitro* modeling of oxidative metabolism have been developed.[2] Although electrochemical oxidation is the most popular method, the UV-induced photocatalytic oxidation in the presence of TiO_2 nanoparticles (UV/TiO_2-PCO), which is widely used for the degradation of pharmaceuticals and other pollutants in wastewater,[3–5] is highly promising for the comprehensive simulation of oxidative metabolism.[6–8]

The oxidation products obtained by UV/TiO_2-PCO are typically identified by mass spectrometry (MS) often coupled to high performance liquid chromatography (HPLC), which introduces pretreatment steps and thus slows down the overall analytical workflow. Therefore, the development of a fast and simple technique of UV/TiO_2-PCO coupled to MS for high-throughput analysis of oxidation products in multiple samples is of considerable current importance. Several sophisticated approaches for the online coupling of UV/TiO_2-PCO to electrospray ionization (ESI) MS have been suggested; they require special instrumentation and additional materials.[9–11] Here, we present a simple, rapid, and cost-effective technique for on-target UV/TiO_2-PCO followed by the direct analysis of oxidation products by matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS). This technique involves the UV irradiation of a microvolume of analyte solution on a TiO_2-covered sample spot of a MALDI target, which serves as a common site for photocatalytic oxidation and MALDI (see Online Supplementary Materials). The use of a TiO_2 layer excludes the need for organic MALDI matrices.

Diclofenac (**1**), a non-steroidal anti-inflammatory drug whose photocatalytic degradation[10] and *in vivo* oxidative bioactivation[13,14] are well known, was used as a test compound. The oxidation products of diclofenac obtained using electrochemical oxidation, conventional photocatalytic oxidation and the suggested on-target photocatalytic oxidation† were analyzed and compared. The analysis of electrochemical oxidation and UV/TiO_2-PCO products was performed using HPLC-MS/MS on a high-resolution FT-ICR instrument.† On the basis of retention times and accurate mass measurements, the molecular structures of oxidation products were proposed (Scheme 1, Table 1 and Online Supplementary Materials).

Diclofenac was detected as the $[M - H]^-$ ion (*m/z* 294), and the base peak at *m/z* 310 was assigned to +O products identified as monohydroxylated derivatives (OH-diclofenac) **2a–c** (Scheme 1). As minor oxidation products, polyhydroxy (**2d–f**,) decarboxylated (**2g–j**), and quinone imine (**3a–d**) derivatives were detected, as well as deep destruction products **4a–f**. Compounds

† For oxidation and analysis procedures, see Online Supplementary Materials.

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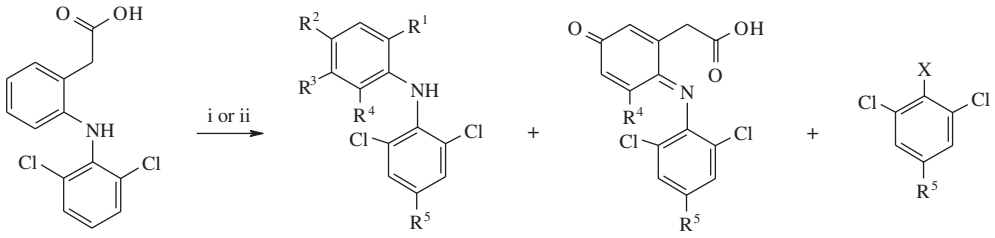

1
diclofenac

2a–j

3a–d

4a–f

	R^1	R^2	R^3	R^4	R^5	Method
2a	CH_2COOH	OH	H	H	H	i, ii
2b	CH_2COOH	H	H	H	OH	ii
2c	CH_2COOH	H	H	OH	H	ii
2d	CH_2COOH	OH	H	H	OH	i, ii
2e	CH_2COOH	OH	H	OH	OH	ii
2f	CH_2COOH	OH	OH	OH	OH	ii
2g	COOH	H	H	H	H	i, ii
2h	CHO	OH	H	H	H	ii
2i	CHO	H	H	H	OH	ii
2j	CHO	OH	H	H	OH	i, ii

	R^4	R^5	Method
3a	H	H	ii
3b	H	OH	i, ii
3c	OH	H	ii
3d	OH	OH	ii

	R^5	X	Method
4a	OH	OH	ii
4b	OH	NH_2	ii
4c	H	NHOH	ii
4d	OH	$COCH_2CH_2OH$	ii
4e	H	$COCH_2COOH$	ii
4f	OH	$COCH_2CH_2COOH$	ii

Scheme 1 *Reagents and conditions:* i, electrochemical oxidation; ii, bulk UV/TiO_2-PCO.

2a, **2d**, **2g, 2j** and **3b** were found among both electrochemical and photocatalytic oxidation products. In the case of UV/TiO_2-PCO, the base peak at *m/z* 310 was assigned to monohydroxylated products **2a–c,** while the peaks at *m/z* 280, 324 and 326 presumably belong to oxidation products **2g, 3b,** and **2d**, respectively. The yield of electrochemical oxidation was relatively low (<20%), while the yield of UV/TiO_2-PCO was much higher, with more than 50% of the total product yield corresponding to OH-diclofenac [Figure 1 (*a*),(*b*)]. These results are in good agreement with published data. For example, the formation of hydroxylated (**2a–d**) and quinone imine (**3a**) oxidation products in the UV/TiO_2-PCO of diclofenac was reported by Calza *et al.*[12] Some discrepancy between the obtained oxidation products may be explained by differences in the experimental parameters such as photocatalytic oxidation time and radiation intensity.

It is generally accepted that two monohydroxylated metabolites arising from diclofenac biotransformation, 4′-OH-diclofenac (**2b**) and 5-OH-diclofenac (**2a**), are involved in the formation of reactive metabolites.[13,14] Thus, compared to electrochemical oxidation, UV/TiO_2-PCO better simulates the oxidative metabolism of diclofenac because it both produces biologically relevant OH-diclofenac metabolites (**2a**,**b**) and provides a higher yield of oxidation products.

Conventional UV/TiO_2-PCO is typically performed in a stirred suspension, and it requires the removal of TiO_2 nanoparticles before the analysis to limit sample throughput and to cause analyte losses due to adsorption on the solid phase. Therefore, in view of the strong UV absorption of TiO_2, which makes it the most promising inorganic matrix for the laser desorption/ionization (LDI) of low molecular weight (LMW) analytes,[13,14] we proposed a simplified workflow with UV/TiO_2-PCO performed directly on a MALDI target and the subsequent MALDI MS analysis. Therefore, TiO_2 nanopowder acts sequentially as a photooxidation catalyst and an LDI matrix.

A thin TiO_2 layer was formed on a MALDI target by drying a droplet of TiO_2 nanopowder suspension within the sample spot. A sample droplet was then applied onto the TiO_2 layer and exposed to UV radiation. The amount of TiO_2 is an important factor affecting the rate of photocatalytic oxidation and the quality of the subsequent MALDI MS analysis. No more than

Table 1 HPLC-MS/MS analysis of electrochemical and photocatalytic oxidation products.

Compound	RT/min	Deprotonated formula $[M - H]^-$	Calculated $[M - H]^-$ (*m/z*)	Found $[M - H]^-$ (*m/z*)	Δ/ppm	OP/diclofenac peak area ratio (%)
Diclofenac	21.94	$C_{14}H_{10}Cl_2NO_2$	294.00831	294.00831	0	42.62
2a	17.14	$C_{14}H_{10}Cl_2NO_3$	310.00322	310.00322	0	11.08
2b	18.76	$C_{14}H_{10}Cl_2NO_3$	310.00322	310.00322	0	11.12
2c	18.06	$C_{14}H_{10}Cl_2NO_3$	310.00322	310.00322	0	35.44
2d	13.93	$C_{14}H_{10}Cl_2NO_4$	325.99814	325.99807	–0.21	1.35
2e	12.20	$C_{14}H_{10}Cl_2NO_5$	341.99305	298.00328 + CO_2	0.20	0.10
2f	6.92	$C_{14}H_{10}Cl_2NO_6$	357.98796	357.98786	–0.27	0.11
2g	15.63	$C_{13}H_8Cl_2NO_2$	279.99266	279.99268	0.42	0.43
2h	19.81	$C_{13}H_8Cl_2NO_2$	279.99266	279.99281	0.53	0.49
2i	20.90	$C_{13}H_8Cl_2NO_2$	279.99266	279.99279	0.46	0.86
2j	16.03	$C_{13}H_8Cl_2NO_3$	295.98757	295.98764	0.23	0.31
3a	24.31	$C_{14}H_8Cl_2NO_3$	307.98757	307.98760	0.09	3.22
3b	14.43	$C_{14}H_8Cl_2NO_4$	323.98249	323.98242	–0.21	1.79
3c	13.13	$C_{14}H_8Cl_2NO_5$	339.97740	339.97721	–0.55	1.21
3d	9.94	$C_{14}H_8Cl_2NO_6$	355.97231	355.97220	–0.30	0.16
4a	7.32	$C_6H_3Cl_2O_2$	176.95046	176.95055	0.50	1.50
4b	9.4	$C_6H_4Cl_2NO$	175.96644	175.96646	0.11	1.10
4c	10.57	$C_6H_4Cl_2NO$	175.96644	175.96650	0.34	0.08
4d	9.1	$C_9H_8Cl_2NO_3$	247.98757	247.98747	–0.40	0.13
4e	12.01	$C_9H_6Cl_2NO_3$	245.97192	245.97180	–0.48	0.07
4f	18.69	$C_{10}H_8Cl_2NO_4$	275.98249	275.98260	0.39	0.68

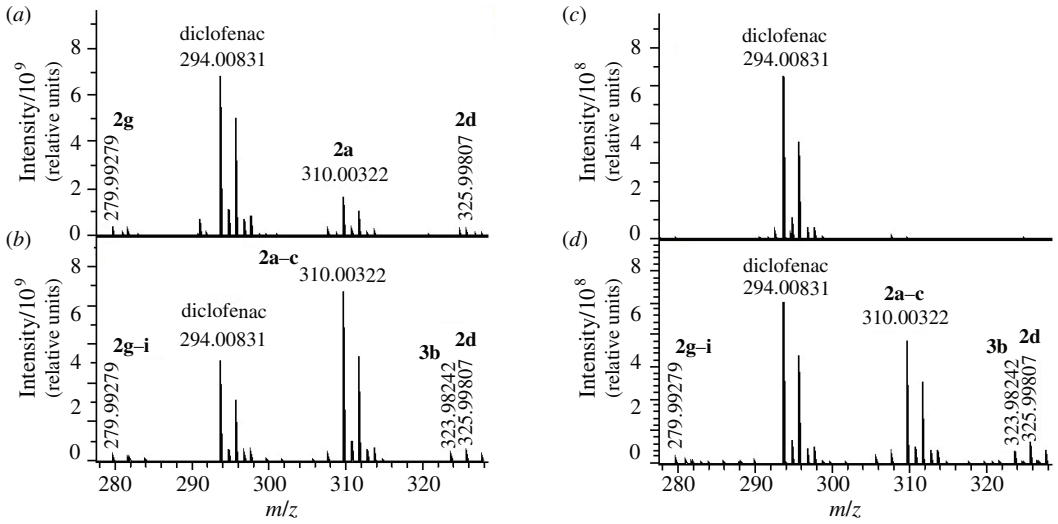

Figure 1 Mass spectra of diclofenac and its oxidation products obtained using ESI-FT-ICR MS after (*a*) electrochemical oxidation, (*b*) bulk UV/TiO_2-PCO, (*c*) MALDI-FT-ICR MS of intact diclofenac and (*d*) after on-target UV/TiO_2-PCO.

0.15 μg of TiO_2 nanopowder (particle size, 21 nm) per sample spot of an MTP 384 MALDI target (~12.5 mm^2) was an optimal amount. The optimal concentration of diclofenac was in a range from 3 to 30 μmol dm^{-3}. Since UV/TiO_2-PCO occurs in a microvolume of the substrate solution, the reaction time is limited by the time of droplet evaporation, which can be easily prolonged by cooling the MALDI target.

We also tested organic matrices commonly used for MALDI of LMW analytes.[17] Optimal signal-to-noise ratios were obtained using TiO_2 *per se* without addition of any organic matrix. This result is consistent with the feasibility of a thin layer of TiO_2 nanopowder as a medium for LDI of LMW compounds, an approach often referred to as surface-assisted LDI (SALDI).[18] The mass spectra obtained in the negative ion mode were easier to interpret because the positive ion mode mass spectra had dominant $[M + Na]^+$ and $[M + K]^+$ peaks, while the $[M + H]^+$ ions were much less abundant.

Figure 1(*d*) shows a typical result of optimized UV/TiO_2-PCO coupled to MALDI-FT-ICR MS. Although not allowing for isomer discrimination, accurate mass measurement demonstrated nearly perfect agreement between the ESI-FT-ICR and MALDI-FT-ICR MS mass spectra of diclofenac oxidation products for conventional [see Figure 1(*b*)] and on-target UV/TiO_2-PCO [see Figure 1(*d*)], respectively. The fact that the base peak in the mass spectra is at *m/z* 310 and minor peaks are at *m/z* 280, 324 and 326 suggests the same oxidation products in both cases.

Similar approaches that couple UV/TiO_2-PCO to ESI MS analysis with different ionization techniques have already been suggested, including on-chip micropillar electrospray ionization (μPESI),[9] desorption electrospray ionization (DESI)[10] and laser ablation electrospray ionization (LAESI).[11] However, these methods require special customized instrumentation and additional materials.

Note that UV/TiO_2-PCO and MS are not coupled online in our approach; therefore, the time-resolved monitoring of the formation of oxidation products to identify the sequential oxidation steps is impossible. Nevertheless, photocatalytic oxidation conditions can be roughly optimized by simply varying the reaction time and UV radiation intensity.

Thus, we have proposed a fast and simple technique for modeling the oxidative metabolism of xenobiotics involving UV/TiO_2-PCO directly on a MALDI target and the subsequent MS analysis of their oxidation products without sample pretreatment. The feasibility of this approach is confirmed by proof-of-concept experiments with diclofenac as a test compound.

This work was supported in part by the Government of the Russian Federation (FASO ISST no. 0074-2014-0012; state registration no. AAAA-A16-116041310010-6). We are grateful to Dr. N. S. Khlebnikova for critically reading the manuscript.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.03.030.

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Received: 28th October 2019; Com. 19/6046