Measurement: Sensors xxx (xxxx) xxx

Contents lists available at ScienceDirect

Measurement: Sensors

journal homepage: www.sciencedirect.com/journal/measurement-sensors

Towards the monitoring of dialysis treatment through absorption and endogenous fluorescence techniques

ARTICLE INFO

Keywords:
Dialysate analysis
Fluorescence spectroscopy
Absorption spectroscopy
Uraemic toxins sensing

ABSTRACT

This study explores the monitoring of uraemic toxins in haemodialysis treatments, beyond urea concentration, in the perspective of reducing complications stemming from the lack of personalized haemodialysis therapies. Absorption and fluorescence techniques were employed to analyse low-middle weight molecules employing laboratory prepared solutions combining p-cresol, bovine albumin and bisphenol-A molecules. These optical techniques offer the advantage of analysing the sample without physical contact, ensuring the potential for implementation in a real-world scenario for continuous treatment monitoring. Real-time monitoring of these toxins in spent dialysate could offer insights into blood concentrations, enhancing dialysis treatment optimization and complication management.

The considered absorption spectra fell within the ultraviolet range (240, 400) nm, while fluorescence spectra were obtained at a selected excitation wavelength of 285 nm. Data analysis revealed a correlation between molecule concentrations and signal intensity in both absorption and fluorescence measurements.

1. Introduction

Haemodialysis is a widely employed technique for patients with kidney disease. Generally, to be monitored urea concentration is evaluated only but, as many works have recently stated [1,2], there exist also other uraemic toxins affecting the treatment effectiveness, namely low middle weight and protein bound solute (PBS) molecules, e.g. organic compounds generated by the intestinal microbiota protein fermentation as free p-cresol, p-cresol/albumin complexes [3] or indoxyl sulphate [4], chemicals as bisphenol-A (BPA), proteins like β_2-microglobulins [5].

Monitoring these molecules enables the optimization of dialytic treatments and reduction of associated complications. Despite these molecules being well-known and easily identifiable in clinical chemistry laboratories, continuous, real time analysis in the patient's blood is a challenging task due to numerous interferents. A potential approach involves monitoring the content of spent dialysate to infer the concentration of these molecules in the blood, considering the characteristics of the dialysis filter and the type of treatment.

This study examines a specific subset of low middle weight molecules and PBS, particularly significant in dialysis treatments, within a set of laboratory prepared solutions combining p-cresol, bovine albumin and BPA using absorption and fluorescence techniques. These optical techniques offer the advantage of analysing the sample without physical contact, ensuring the potential for implementation in a real world scenario for continuous treatment monitoring, through designing an optoelectronics sensor placed across the spent dialysate line.

The measurements were performed by a laboratory spectrofluorometer (Jasco FP6200) and spectrophotometer (UV–Vis Jasco V-570), facilitating comprehensive measurements encompassing both absorption and fluorescence emission/excitation spectra.

Given the organic nature of the toxins of interest, our study focused on the spectral region within the ultraviolet range, i.e. UVA-UVB: (240, 400) nm, for absorption and excitation of endogenous fluorescence.

This paper is structured as follows: Section 2 details the rationale behind molecule selection, the preparation of laboratory solutions, and the acquisition mode for measurements. The results of the spectral analysis are discussed in Section 3, and Section 4 outlines future improvements and ongoing work.

2. Methods and procedures

2.1. Molecules selection

A set of solutions was prepared to study the absorption and endogenous fluorescence characteristics of the molecules of interest (MoI). The selection of MoI comprises toxins closely associated with the dialysis treatment process.

P-cresol is a protein bound uraemic toxin of great interest for its involvement in endothelial damage, neurologic and cardiovascular complications. Together with its conjugate derivatives, such as p-cresyl sulphate, it has not been accurately investigated yet, albeit being marked as uraemic toxin. Therefore, its relative optical properties are still under examination. This is a significant lack of information considering that, for instance, authors of [6] attribute to free p-cresol cardiovascular issues in non-diabetic dialytic patients. Another notable evidence is reported in Ref. [7], providing a literature review that underlines the hypothesis of a role of p-cresol, its conjugate derivate p-cresyl sulphate and indoxyl sulphate in colon cancer development and progression in chronical kidney disease (CKD) patients.

When kidney function is compromised, an array of substances accumulates within the body, with certain ones forming bonds with the transport protein human serum albumin (HSA). These substances,

https://doi.org/10.1016/j.measen.2024.101331

pivotal in both contributing to and indicating the presence of uraemic syndrome, subsequently transition into uraemic toxins [8].

BPA is the main component of several plastics because it confers rigidity, resistance, and transparency. It is also a component of some dialysis membranes, and it accumulates in patients affected by chronic kidney disease. Observational studies have linked BPA exposure to kidney and cardiovascular injury in humans and to the activation of carcinogenesis pathways [9]. If healthy individuals can rapidly excrete BPA, patients with chronic kidney disease may show higher and potentially dangerous concentrations [10].

In conclusion, the identified MoI are: (i) p-cresol, (ii) albumin and (iii) BPA.

2.2. Solutions preparation

Three stock solutions with the following concentrations were predisposed, with a base of distilled water (ddH2O): p-cresol 1 mg/mL; bovine albumin 1 mg/mL; BPA 100 μg/mL. From the stock solutions, 7 solutions of 10 mL volume were prepared with the following concentrations: p-cresol 1 μg/mL, 0.01 mg/mL and 0.1 mg/mL; albumin 0.01 mg/mL and 0.1 mg/mL; BPA 0.1 μg/mL and 5 μg/mL. Besides the single MoI analysis in 3 mL samples, from each solution a set of 4 solutions mixing the three molecules, still with volume 3 mL was prepared. These samples mirror what is typically found in dialysate during a typical dialysis treatment. They were chosen to observe the combined effects and interactions among the different MoIs.

The concentrations of each solute within these solutions are detailed in Table 1.

2.3. Measurements procedure

For each prepared sample, a series of measurements was conducted using the UV–Vis spectrophotometer Jasco V-570 and the spectrofluorometer Jasco FP6200. From the analysis of the absorption spectra, we identified a potential excitation wavelength to stimulate endogenous fluorescence at $\lambda_{exc} = 285$ nm, which also appears to be particularly suitable for monitoring p-cresol.

3. Results and discussion

3.1. Absorption spectra

The absorption spectra obtained with the spectrophotometer were firstly acquired for the samples containing only one MoI. The results, properly compensated to the reference absorption spectrum from ddH2O, can be seen in Fig. 1. As is possible to notice in Fig. 1a, p-cresol shows the highest absorbance variation as the concentration increases. The absorption spectra of albumin, as shown in Fig. 1b, and that of BPA in Fig. 1c, exhibit a relatively low contribution within the considered spectral range.

The albumin concentration in spent dialysate resulted in

Table 1
MoI: prepared solutions concentrations.

P-cresol	Albumin	BPA
0.1 mg/mL	–	–
0.01 mg/mL	–	–
1 μg/mL	–	–
–	0.01 mg/mL	–
–	0.1 mg/mL	–
–	–	0.1 μg/mL
–	–	5 μg/mL
3.3 μg/mL	3.3 μg/mL	33 ng/mL
0.33 μg/mL	3.3 μg/mL	33 ng/mL
3.3 μg/mL	33 μg/mL	33 ng/mL
3.3 μg/mL	3.3 μg/mL	1.67 μg/mL

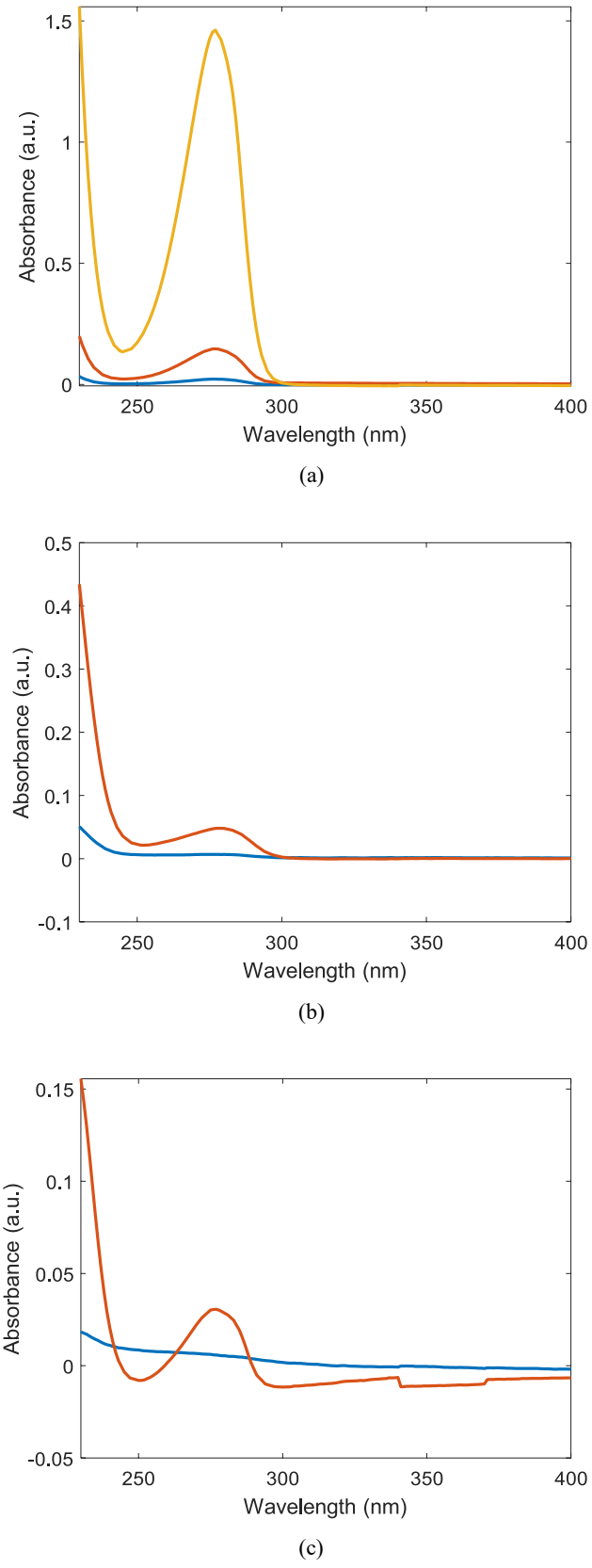


Fig. 1. (a) p-cresol absorption spectra, with concentrations: 1 μg/mL (—), 0.01 mg/mL (—), 0.1 mg/mL (—). (b) Albumin absorption spectra, with the following concentrations: 0.01 mg/mL (—), 0.1 mg/mL (—). (c) BPA absorption spectra, with the following concentrations: 0.1 μg/mL (—), 5 μg/mL (—).

approximately 0.024 mg/mL, after being measured in samples provided by the Laboratory of Nephrology (CHIMOMO Department, University of Modena and Reggio Emilia, Modena, Italy), allowed by the AVEN Ethical Committee. As a consequence, the maximum concentration of albumin in the solutions used in this study represents a worst case scenario compared to what is typically encountered in a dialysis treatment. Despite BPA showing absorption near 285 nm, the concentrations used in this study greatly exceeds typical levels found in healthy or pathological conditions, which are usually in the range of a few ng/mL as noted by Gonzalez et al. (2013) [11]. Therefore, in a realistic scenario, the predominant absorption contribution in the considered spectral range is attributed to p-cresol. This is further supported by the highest sensitivity to p-cresol at $\lambda_{exc} = 285$ nm, as shown in Table 2. In Fig. 2, the absorbance of the mixed samples is shown. Again, the absorbance significantly decreases only when p-cresol concentration decreases from 3.3 μg/mL to 0.33 μg/mL. The other results, coming from an increase of albumin or BPA, keep similar values as also resumed in Table 3.

3.2. Fluorescence spectra

For fluorescence measurements, the excitation wavelength was chosen as $\lambda_{exc} = 285$ nm, close to the absorption peak of p-cresol. The exact wavelength of the p-cresol peak absorption, i.e., 280 nm, was not chosen because, for the development of an optoelectronics sensor, 285 nm was found to better align with the peak wavelengths of commercially available LED sources.

The emission spectra were acquired in the spectral range (300–450) nm. As for the absorption measurements, the results were properly compensated to the reference fluorescensce emission spectrum obtained from ddH2O.

The samples containing only one MoI were firstly analysed obtaining the spectra shown in Fig. 3. As is possible to notice in Fig. 3a, p-cresol is the molecule emitting the most endogenous fluorescence, with a peak emission at approximately $\lambda_{em\ p-cresol} = 307$ nm. The plot corresponding to the maximum p-cresol concentration clearly saturates, due to the spectrofluorometer full-scale limitations. As a consequence, it has not been considered in the sensitivity computation, whose results are represented in Table 4. Albumin fluorescence emission spectra are shown in Fig. 3b. It can be seen that the sample with the highest concentration exhibits a lower emission at approximately $\lambda_{em\ p-cresol} = 307$ nm with respect to the p-cresol corresponding one, at comparable concentrations. Furthermore, it can be observed that also the fluorescence emission peak of albumin that occurs at approximately $\lambda_{em\ albumin}$ 350 nm has a lower intensity than p-cresol. Lastly, the BPA endogenous fluorescence appeared very low, albeit referred to much lower concentrations than the other two MoI, as shown in Fig. 3c. Moreover, its fluorescence exhibited a low value at the highest concentration.

Lastly, as in Section 3.1, a further confirmation of the highest sensitivity to p-cresol for an emission wavelength $\lambda_{em} = 307$ nm excited at $\lambda_{exc} = 285$ nm, can be seen in Table 4. Again, by computing the angular coefficient still derived from the linear interpolation between concentrations and the related fluorescence emission, the sensitivity is much higher for p-cresol compared to albumin and BPA. As previously mentioned, due to the saturation obtained with the sample with the highest p-cresol concentration, the related sensitivity value has been computed by using only the minimum and medium concentration values, i.e. 1 μg/mL and 0.01 mg/mL. In Fig. 4, the fluorescence

Table 2
Sensitivity of absorption at $\lambda_{exc} = 285$ nm to the concentration of individual MoIs.

Molecule	Sensitivity (mL/mg)
P-cresol	9.7274
Albumin	0.3918
BPA	2.9606

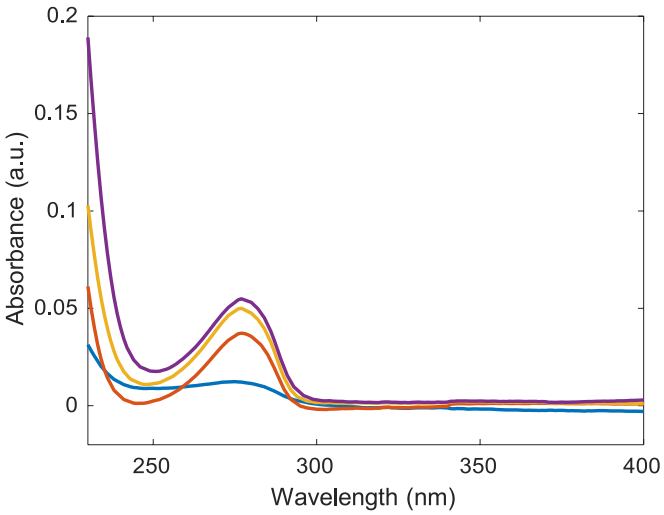


Fig. 2. p-cresol mixed with albumin and BPA absorption spectra, with the following concentrations: p-cresol 0.33 μg/mL + albumin 3.3 μg/mL + BPA 33 ng/mL (—), p-cresol 3.3 μg/mL + albumin 3.3 μg/mL + BPA 33 ng/mL (—), p-cresol 3.3 μg/mL + albumin 3.3 μg/mL + BPA 1.67 μg/mL (—), p-cresol 3.3 μg/mL + albumin 33 μg/mL + BPA 33 ng/mL (—).

Table 3
Absorbance corresponding to $\lambda_{exc} = 285$ nm for each solution combining all four molecules. The mixed sample row implies the following concentrations: p-cresol 3.3 μg/mL + albumin 3.3 μg/mL + BPA 33 ng/mL. The subsequent rows in the table indicate the observed absorption (second column) by varying the concentration of each molecule to the value reported in the first column.

Solution	Absorbance @285 nm (a.u.)
Mixed sample	0.02622
P-cresol 0.33 μg/mL	0.00901
Albumin 33 μg/mL	0.04193
BPA 1.67 μg/mL	0.03635

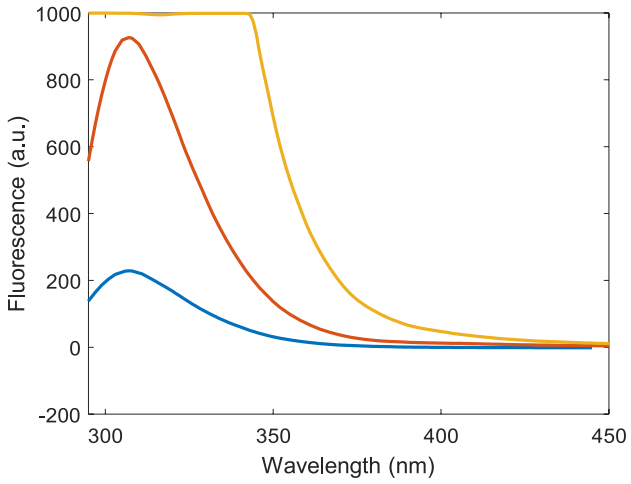
emission spectra of the mixed samples are shown. The fluorescence significantly decreases only when p-cresol concentration decreases from 3.3 μg/mL to 0.33 μg/mL. The other results, coming from an increase of albumin or BPA, keep similar values as also resumed in Table 5.

4. Conclusions

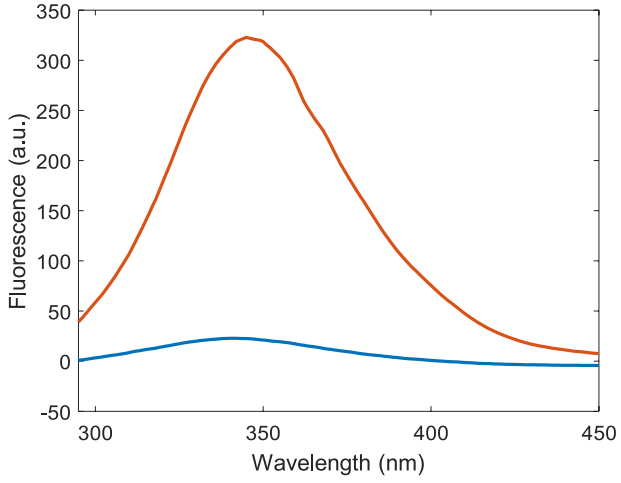
In this paper, the absorption and endogenous fluorescence properties of MoI were investigated. The discussed results show that p-cresol exhibits a significantly higher absorption within the considered wavelength range compared to the other investigated molecules. This translates into a greater sensitivity of absorption with varying concentrations of this molecule. By fixing an excitation wavelength at 285 nm, i.e., available among commercial LED emissions, we observed endogenous emission in the spectral range (300–450) nm for all three considered molecules. With increasing concentration, the molecule exhibiting the highest emission is p-cresol followed by albumin, while BPA shows extremely reduced emission. It should be noted that the emission peaks of p-cresol and albumin are shifted by approximately 40 nm.

From the analysis reported in this paper, it seems reasonable to hypothesize the possibility of combining absorption and fluorescence information to distinguish these molecules. Monitoring all these molecules could facilitate the optimization of dialytic treatments and reduction of associated complications; however, the high sensitivity observed in terms of both absorption and fluorescence to p-cresol suggests a simpler development of a sensor for monitoring this molecule.

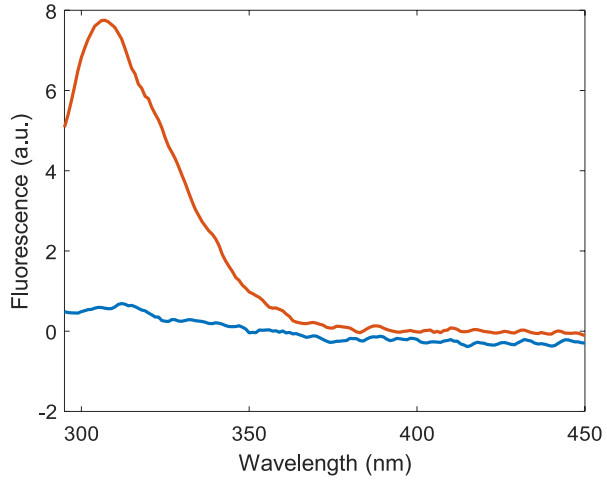
Nonetheless, developing a sensor entails significant additional



(a)



(b)



(c)

Fig. 3. (a) p-cresol fluorescence emission spectra, with concentrations: 1 μg/mL (—), 0.01 mg/mL (—), 0.1 mg/mL (—). (b) Albumin fluorescence emission spectra, with the following concentrations: 0.01 mg/mL (—), 0.1 mg/mL (—). (c) BPA fluorescence emission spectra, with the following concentrations: 0.1 μg/mL (—), 5 μg/mL (—).

Table 4
Single molecules fluorescence sensitivity.

Molecule	Sensitivity (mL/mg)
P-cresol	71646.1
Albumin	927.0
BPA	1844.4

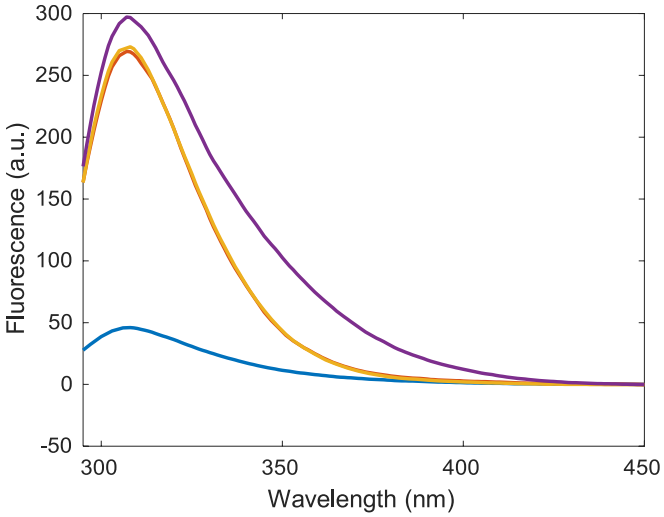


Fig. 4. p-cresol mixed with albumin and BPA fluorescence emission spectra, with $\lambda_{exc} = 285$ nm, with the following concentrations: p-cresol 0.33 μg/mL + albumin 3.3 μg/mL + BPA 33 ng/mL (—), p-cresol 3.3 μg/mL + albumin 3.3 μg/mL + BPA 33 ng/mL (—), p-cresol 3.3 μg/mL + albumin 3.3 μg/mL + BPA 1.67 μg/mL (—), p-cresol 3.3 μg/mL + albumin 33 μg/mL + BPA 33 ng/mL (—).

Table 5
Fluorescence corresponding to $\lambda_{em} = 307$ nm and $\lambda_{exc} = 285$ nm for each solution combining all four molecules. The mixed sample row implies the following concentrations: p-cresol 3.3 μg/mL + albumin 3.3 μg/mL + BPA 33 ng/mL. The subsequent rows in the table indicate the observed fluorescence (second column) by varying the concentration of each molecule to the value reported in the first column.

Solution	Fluorescence @307 nm (a.u.)
Mixed sample	270.9
P-cresol 0.33 μg/mL	46.0
Albumin 33 μg/mL	297.2
BPA 1.67 μg/mL	274.4

preliminary work. Other molecules known to be present in dialysate should also be considered and a spectrofluorimetric analysis of real dialysate samples acquired at different stages of treatment should be studied. Nevertheless, we believe that the preliminary results reported in this paper are encouraging and can pave the way for further studies.

Funding statement

The research leading to these results has received funding from the European Union - NextGenerationEU through the Italian Ministry of University and Research under PNRR - M4C2-I1.3 Project PE_00000019 "HEAL ITALIA" to Gabriele Donati, CUP E93C22001860006.

The views and opinions expressed are those of the authors only and do not necessarily reflect those of the European Union or the European Commission. Neither the European Union nor the European Commission can be held responsible for them.

Acknowledgements

The authors would like to thank LADAC laboratory, in particular Barbara Zanfrognini and Monica Malavasi for making possible the spectrophotometer and spectrofluorometer usage.

References

[1] J. Arund, M. Luman, F. Uhlin, R. Tanner, I. Fridolin, Is fluorescence valid to monitor removal of protein bound uremic solutes in dialysis? PLoS One 11 (2016) e0156541, 05.
[2] R. Vanholder, A. Pletinck, E. Schepers, G. Glorieux, Biochemical and clinical impact of organic uremic retention solutes: a comprehensive update, Toxins 10 (2018) 33, 01.
[3] B. Bammens, P. Evenepoel, H. Keuleers, K. Verbeke, Y. Vanrenterghem, Free serum concentrations of the proteinbound retention solute p-cresol predict mortality in hemodialysis patients, Kidney Int. 69 (6) (2006) 1081–1087.
[4] J. Holmar, J. Arund, F. Uhlin, R. Tanner, I. Fridolin, Quantification of indoxyl sulphate in the spent dialysate using fluorescence spectra, in: 15th Nordic-Baltic Conference on Biomedical Engineering and Medical Physics (NBC 2011) 14- 17 June 2011, Springer, Aalborg, Denmark, 2011, pp. 45–48.
[5] K. Lauri, J. Arund, J. Holmar, R. Tanner, S. Kalle, M. Luman, I. Fridolin, Removal of urea, β2-microglobulin, and indoxyl sulfate assessed by absorbance and fluorescence in the spent dialysate during hemodialysis, Am. Soc. Artif. Intern. Organs J. 66 (6) (2020) 698–705.
[6] B. Meijers, B. Bammens, B. De Moor, K. Verbeke, Y. Vanrenterghem, P. Evenepoel, Free p-cresol is associated with cardiovascular disease in hemodialysis patients, Kidney Int. 73 (10) (2008) 1174–1180.
[7] R. Di Paola, A. De, R. Izhar, M. Abate, S. Zappavigna, A. Capasso, A.F. Perna, A. La Russa, G. Capasso, M. Caraglia, et al., Possible effects of uremic toxins p-cresol, indoxyl sulfate, p-cresyl sulfate on the development and progression of colon cancer in patients with chronic renal failure, Genes 14 (6) (2023) 1257.
[8] F. Zare, A. Janeca, S.M. Jokar, M. Faria, M.C. Gonçalves, Interaction of human serum albumin with uremic toxins: the need of new strategies aiming at uremic toxins removal, Membranes 12 (3) (2022) 261.
[9] B. Quiroga, Strategies to protect dialysis patients against bisphenol a, Biomolecules 11 (9) (2021) 1375.
[10] E. Bosch-Panadero, S. Mas, D. Sanchez-Ospina, V. Camarero, M.V. Pérez-Gómez, I. Saez-Calero, P. Abaigar, A. Ortiz, J. Egido, E. González-Parra, The choice of hemodialysis membrane affects bisphenol a levels in blood, J. Am. Soc. Nephrol. 27 (5) (2016) 1566–1574.
[11] E. González-Parra, J.A. Herrero, U. Elewa, R.J. Bosch, A.O. Arduán, J. Egido, et al., Bisphenol a in chronic kidney disease, Int. J. Nephrol. 2013 (2013).

Valentina Di Pinto[a,*], Gioia Galli[a], Giovanni Gibertoni[a], Alberto Ferrari[a], Marco Ferrarini[b], Niccolò Morisi[b], Silvia Giovanella[b], Giulia Ligabue[b], Gabriele Donati[b], Luigi Rovati[a]

[a] *University of Modena and Reggio Emilia, "Enzo Ferrari" Engineering Department, Modena, Italy*

[b] *University of Modena and Reggio Emilia, CHIMOMO Department, Modena, Italy*

[*] Corresponding author.

E-mail addresses: valentina.dipinto@unimore.it (V. Di Pinto), 254083@studenti.unimore.it (G. Galli), giovanni.gibertoni@unimore.it (G. Gibertoni), alberto.ferrari8@unimore.it (A. Ferrari), marco.ferrarini@unimore.it (M. Ferrarini), niccolo.morisi@unimore.it (N. Morisi), silvia.giovanella@unimore.it (S. Giovanella), giulia.ligabue@unimore.it (G. Ligabue), gabriele.donati@unimore.it (G. Donati), luigi.rovati@unimore.it (L. Rovati).