Journal of Electroanalytical Chemistry 973 (2024) 118666

Contents lists available at ScienceDirect

Journal of Electroanalytical Chemistry

journal homepage: www.elsevier.com/locate/jelechem

Dual electrochemical detection of leucovorin and its metabolite 5-methyltetrahydrofolic acid

Pui Hang Shum, Lynn Dennany *

WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, Technology and Innovation Centre, 99 George Street, Glasgow G1 1RD, UK

ARTICLE INFO

Keywords:
Leucovorin
5-methyltetrahydrofolate
Precision detection
Electroanalytical detection
SWV

ABSTRACT

Current healthcare trends focus on personalised precision medicine, which customises treatment based on individual responses to both diseases and therapeutic interventions. This marks a departure from a "one size fits all" approach towards a more sophisticated strategy. However, notwithstanding progress in the theoretical knowledge for personalised precision approaches, resource constraints impede their implementation. Monitoring drug therapies is vital for the advancement of precision medicine, alongside drug development and targeted treatment strategies. This study presents the potential of electrochemical approaches including square wave voltammetry (SWV) as a proof-of-concept for the simultaneous monitoring of circulating concentrations of 5-methyltetrahydrofolate (5-MTHF) and leucovorin calcium (LV) within biological matrices. An easy-to-use and portable sensor has been developed for the detection of 5-MTHF and LV at therapeutically relevant concentrations without prior sample preparation. The sensor was successfully tested in artificial urine and human pooled serum, demonstrating its efficacy in diverse biological matrices. This approach successfully illustrated the dual detection of LV and 5-MTHF over the linear range 0 to 10 μM which is within the therapeutically relevant range for both within urine. The developed sensor exemplifies the potential of electrochemical sensors as point-of-care devices. Its rapid time to result and elimination of time-consuming sample preparation improve usability for clinicians, broadening the application of electrochemical sensors in clinical settings. This dual monitoring approach offers significant contributions to the evolution of precision medicine by providing real-time, accurate drug monitoring capabilities.

1. Introduction

Leucovorin calcium (LV), also known as folinic acid, is a medication known for enhancing the efficacy of certain chemotherapy drugs, mitigating the toxic effects of methotrexate (MTX), and managing folate deficiency [1–4]. It is a chemically reduced form of folic acid that bypasses the blocked dihydrofolate reductase enzyme (DHFR), replenishing the active folate pool in the body, which allows for DNA synthesis and repair [5–7]. Originally developed to counter the harmful effects of folic acid antagonists such as MTX, LV's ability to rescue normal cells from toxicity marked a significant advancement in cancer therapy [8,9]. In chemotherapy, LV is widely used as an adjuvant, particularly with fluorouracil (5-FU), enhancing the drug's binding to thymidylate synthase and increasing its anti-tumour activity [10].

Administered either orally, intramuscularly, or intravenously depending on the clinical scenario, LV's dosage varies based on its use, ranging from small doses for MTX rescue to larger amounts in combination with chemotherapy agents like 5-FU. Generally well-tolerated, LV's potential side effects include allergic reactions, gastrointestinal disturbances, and rare instances of seizures. Therefore, careful monitoring is essential, especially with high doses or when combined with potent chemotherapeutic agents, to ensure patient safety and treatment efficacy.

LV and its primary metabolite, 5-methyltetrahydrofolic acid, (5-MTHF) are two essential folate derivatives that play crucial roles in various metabolic processes within the human body (see Fig. 1). The detection and quantification of these compounds are of significant importance in clinical settings, particularly in the context of pharmacokinetics and chemotherapy condition [11–14].

The simultaneous monitoring of LV and 5-MTHF levels is crucial for several reasons. During chemotherapy regimens, LV is administered in combination with certain chemotherapeutic agents to counteract their toxic effects and enhance their efficacy. Therefore, monitoring of LV levels is essential for optimizing the therapeutic efficacy and minimizing

* Corresponding author.
E-mail address: lynn.dennany@strath.ac.uk (L. Dennany).

https://doi.org/10.1016/j.jelechem.2024.118666
Received 23 July 2024; Received in revised form 16 September 2024; Accepted 18 September 2024
Available online 22 September 2024

potential side effects. Concurrently, the measurement of 5-MTHF levels can provide valuable insights into an individual's folate status, which is linked to various health conditions, such as neural tube defects, cardiovascular diseases, and certain types of cancer [15,16]. Furthermore, the interplay between LV and 5-MTHF is complex, as LV can be metabolized to other folate derivatives, including 5-MTHF. Therefore, the simultaneous detection of both compounds can provide a more comprehensive understanding of their metabolic relationships and pharmacokinetic profiles. In certain clinical scenarios, such as during high-dose MTX therapy, the co-administration of LV and 5-MTHF is recommended to ensure optimal rescue and prevent potentially life-threatening toxicities. Simultaneous monitoring of both compounds is essential for tailoring treatment regimens and optimizing patient outcomes.

A previous study has worked on monitoring of methotrexate (MTX), LV and their metabolites, which include 5-MTHF, using capillary electrophoretic method. This studied the metabolite pattern in those patients with delayed elimination or signs of toxicity for specific features which might allow an early diagnosis and treatment [15].

Traditional analytical techniques, such as high-performance liquid chromatography (HPLC) and immunoassays, have been utilised to simultaneously monitor MTX, LV and 5-MTHF [15], however, these often require complex sample preparation, specialized instrumentation, and skilled personnel, hindering their widespread implementation for point-of-care (POC) testing. In contrast, electrochemical detection methods, particularly square wave voltammetry (SWV), offer several advantages, including simplicity, cost-effectiveness, and the potential for miniaturization and POC testing [17–21]. Electrochemical techniques including SWV and electrochemiluminescence (ECL) have previously been shown to effectively detect and quantify LV in simulated biological samples [17,22].

The ability to perform simultaneous analysis of multiple analytes in a single sample using electrochemical techniques makes it a promising approach for the concurrent detection of LV and 5-MTHF, enabling more efficient monitoring and personalized treatment strategies. By addressing the limitations of existing techniques and providing a POC testing solution for the simultaneous detection of leucovorin and 5-MTHF, this research could facilitate improved patient care, treatment optimization, and better management of folate-related conditions. Herein, we describe the dual detection of LV and 5-MTHF in artificial urine and human pooled serum as an initial proof-of-concept towards assessing this approach for POC monitoring of cancer therapies.

2. Materials and methods

2.1. Materials

Leucovorin calcium (LV), 5-methyltetrahydrofolate (5-MTHF), along with all the constituents for artificial urine, outlined previously [17], and human pooled serum were purchased from Sigma Aldrich. Tri-sodium citrate dihydrate, creatinine, ammonium chloride, potassium oxalate monohydrate and sodium sulfate were purchased from Thermo Fisher Scientific. Sodium chloride was purchased from VWR. Urea was purchased from GE Healthcare Life Sciences. Artificial saliva (Bioténe® oral balance gel) was commercially purchased and used as received. All chemicals were used as received and all solutions prepared in Milli-Q water (18 MΩ cm^{-1}).

2.2. Instrumentation

All electrochemical measurements were performed with a PalmSens EmStat Blue potentiostat utilising carbon screen printed electrodes (SPCE) as previously described [17] Electrochemical measurements were performed using SPCE electrodes (SPCE DRP-110) purchased from Metrohm (UK) with a 4 mm diameter carbon working electrode, a carbon paste counter electrode and an Ag paste quasi-reference electrode. All electrochemical analysis was performed at room temperature with cyclic voltammetric analysis performed over the potential range $0 \leq E \leq 1.5$ V versus an Ag paste quasi-reference, at a scan rate of 100 mVs^{-1}. Square wave voltammetry (SWV) parameters stated pulse amplitude 100 mV and frequency of 50 Hz.

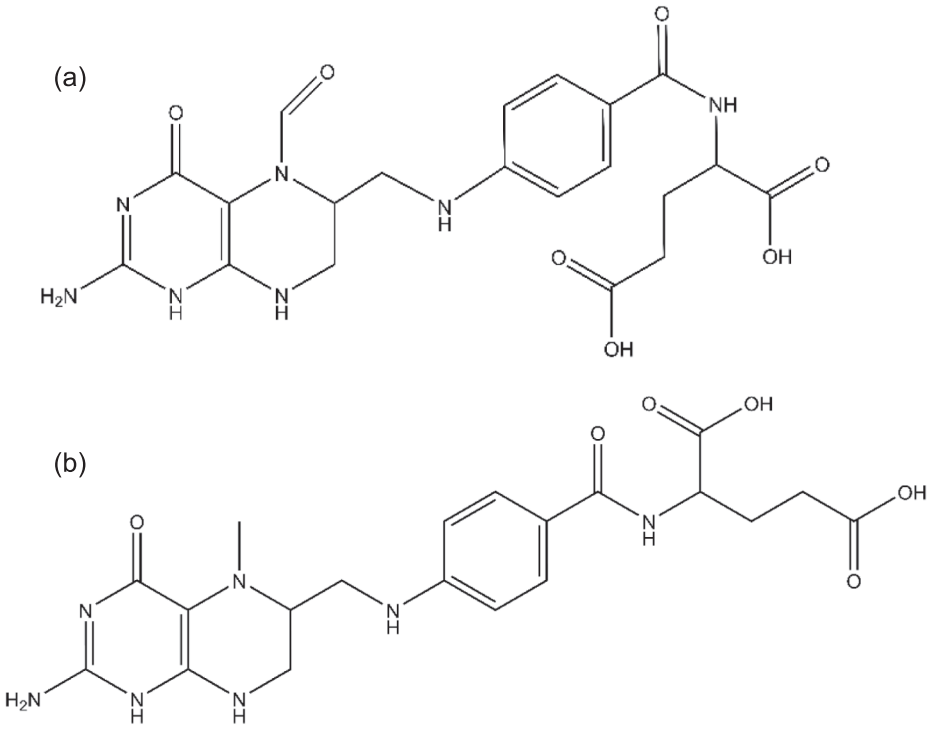

Fig. 1. Molecular structure of (a) LV and (b) 5-MTHF.

2.3. Sample preparation of LV and 5-MTHF

LV was diluted with 1:9 EtOH: 0.1 M NaCl (v/v) ratio to obtain varying concentration ranges (7.5 – 20 μM). 5-MTHF was dissolved in 1:9 EtOH: 0.1 M NaCl (v/v) ratio to obtain varying concentration ranges (8.16 – 21.75 μM representing 3.75–8.75 μg/mL). For biological matrix samples, LV and 5-MTHF were diluted with 1:9 EtOH: 0.1 M NaCl (v/v) ratio and spiked as previously outlined [17]. All samples were stored at 2 – 8 °C. For pH adjustments 1 M NaOH or HCl were used as required.

2.4. Preparation of artificial urine samples

Artificial urine was prepared as previously described with 11.965 mM Na_2SO_4, 1.487 mM $C_5H_4N_4O_3$, 2.45 mM $Na_3C_6H_5O_7{\cdot}2H_2O$, 7.791 mM $C_4H_7N_2O$, 21.619 mM KCl, 39.387 mM NaCl, 1.663 mM $CaCl_2$, 23.667 mM NH_4Cl, 0.19 mM $K_2C_2O_4{\cdot}7H_2O$, 4.389 mM $MgSO_4{\cdot}7H_2O$, 18.667 mM $NaH_2PO_4{\cdot}2H_2O$, and 4.667 mM $K_2HPO_4{\cdot}2H_2O$ in 1000 mL [17].

3. Results and discussion

3.1. Electrochemical characterisation

The electrochemical characterisation of LV and 5-MTHF has been reported in the past studies [23–27]. The resultant CV observed in this contribution showed two anodic peaks at ~0.25 V and 1.2 V for 5-MTHF (Fig. 2) and two anodic peaks at ~0.7 and ~1.26 V for LV (see Fig. S1) which agrees with previous work [17]. This dual anodic wave system is very similar to that described for LV which only proceeds via a two-step process and is in line with the proposed mechanism for the electrochemical oxidation of folic acid which has been shown to involve the nitrogen from the amino groups that can be oxidised, which for 5-MTHF this would result in the formation of the oxidation product 5-methyltetrahydrofolate (5-MDHF) [17,22,23,28–31]. In lieu of the fact that 5-MTHF is similar in structure to 5,8-dihydrofolic acid, it can be concluded that 5-MTHF may also have an irreversible redox process under these conditions due to the same tautomerisation effect [22]. At the potential of the first oxidation peak, one electron is removed to form a stable cation radical in which the spin is delocalised over nine nuclei. The second oxidation of this cation radical requires more energy and therefore occurs at a higher potential (~1.2 V).

As with previous work undertaken on LV, the concentrations needed for observable CV responses was in excess of the therapeutic and clinically relevant concentrations ranges typically utilized for patient treatment, therefore SWV was utilized.

Fig. 3 shows a typical SWV of 5-MTHF with the observable peaks being consistent with the CV response and previous work in literature. Following on from this previous work, this contribution utilized the optimal scan rate [17]. It is acknowledged that scan rate would impact on the response, too fast might result in poor separation of the peaks observed and too slow would make the sensor unfeasible. In addition, in keeping with the SWV detection of LV, the second anodic peak at ~1.2 V is considerably smaller compared to the peak at ~0.3 V. The 1.2 V peak is also observed for LV, and it has been shown that 5-MTHF when exposed to air, will produce a third oxidation peak between these two main peaks with a corresponding reduction in the 1.2 V peak [23]. This degradation could be avoided if analysis was performed under nitrogen conditions at pH 5. However, to ensure minimal sample preparation and analysis steps for POC detection, it was determined that detection and quantification of 5-MTHF would be based upon the 0.3 V anodic peak.

3.2. Analytical parameters

pH can play a significant role in the electrochemical behaviour of an analyte and can therefore have an important impact on the design of an analytical sensor, particularly important for biosensors focusing on detection within biological matrices [32–36]. Previous research on LV has established that the optimal pH for its detection is pH 5 and shown its independent detection [17]. pH 7 was chosen to demonstrate detection performance at a physiological pH in comparison to the optimal at pH 5. Previous work has shown the detection of LV at pH 5 and pH 7 [17]. Given the structurally similarities of LV and 5-MTHF it is likely that 5-MTHF pK_as are associated with the glutamyl carboxylic acid moieties and the hydroxyl group on the amine functional group. In addition, as the ultimate aim is to detect both LV and 5-MTHF simultaneously within biological matrices and previous work has shown that the optimal detection of LV is at pH 5, the concentration dependence of 5-MTHF was examined at both pH 5 and pH 7. These were chosen to elucidate the optimal conditions for both qualitative and semi-quantitative analysis.

Fig. 4 (a) and (b) illustrate the electrochemical behaviour of 5-MTHF at pH 5 and pH 7 at various concentrations. In agreement with data presented previously for LV, at these much lower clinically relevant concentrations, the first oxidation peak is observed for 5-MTHF. Unlike LV, which showed a negligible difference in detection at pH 5 and 7 [17], the response for 5-MTHF is much more dependent on pH, with the

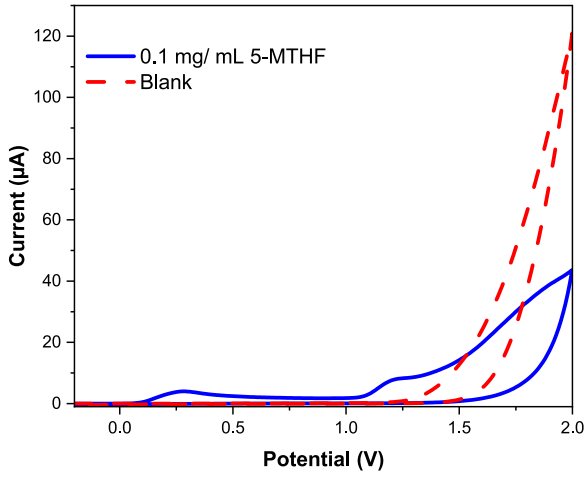

Fig. 2. Typical CV of 0.1 mg mL^{-1} 5-MTHF (blue solid line) in EtOH: 0.1 M NaCl aq (1:9 v/v) and EtOH: 0.1 M NaCl (1:9 v/v) (red dashed line) collected from an unmodified SPCE at a scan rate of 100 mV/s across a potential range of $-0.20 \leq E \geq 2.0$ V vs Ag.

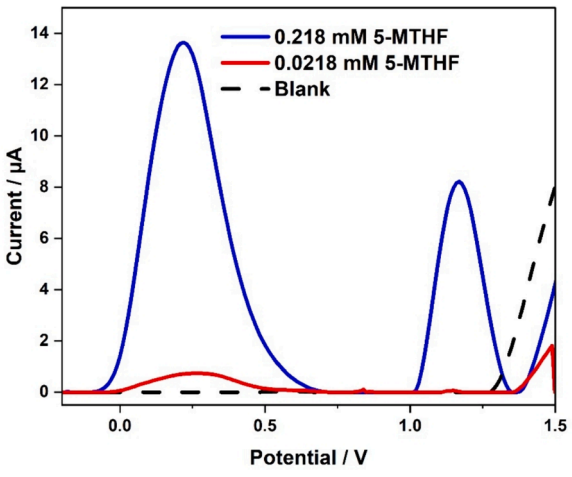

Fig. 3. Typical SWV of 0.1 mg mL^{-1} 5-MTHF (blue solid line) in EtOH: 0.1 M NaCl aq (1:9 v/v) and EtOH: 0.1 M NaCl (1:9 v/v) (red dashed line) collected from an unmodified SPCE scanned over the potential range $-0.20 \leq E \leq 1.5$ V vs Ag at pulse amplitude of 100 mV and frequency of 50 Hz.

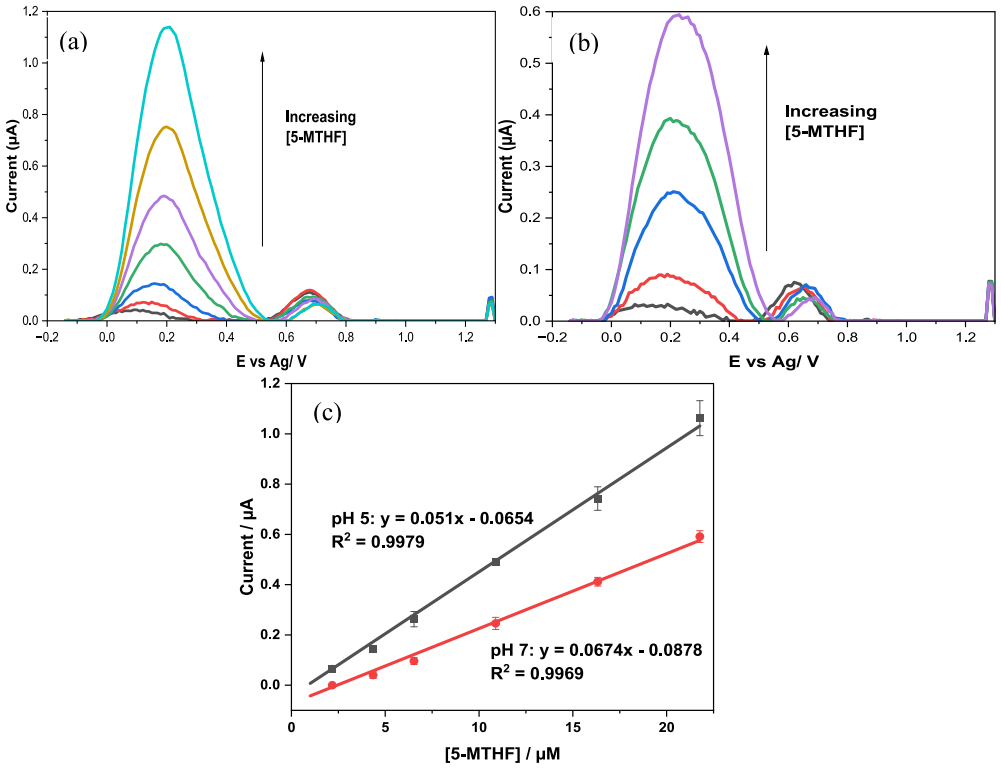

Fig. 4. Typical SWV for increasing [5-MTHF] in EtOH: 0.1 M NaCl aq (1:9 v/v) at (a) pH 5 and (b) pH 7 over the concentration range 0 ≤ [5-MTHF] ≤ 21.76 μM scanned over the potential range 0 ≤ E≤1.3 V vs Ag at a pulse amplitude of 100 mV and frequency of 50 Hz. (c) Typical trend of maximum i_p at 0.2 V at (grey squares) pH 7 and (red circles) pH 5. Error bars represent the standard deviation obtained from triplicate results.

response for pH 5 being almost double that observed for pH 7, indicating that the detection of 5-MTHF is much more effective at pH 5.

For the simultaneous detection of 5-MTHF and LV, two calibrations were obtained at pH 7 and pH 5, reflecting the pH values commonly encountered for urine (pH 5) and serum or blood (pH 7). As illustrated in Fig. 4 (a) and (b), the peak at ~0.2 V increased linearly with increasing [5-MTHF], however, the peak current observed for the response at pH 5 is almost double that of pH 7. This is consistent with previous work for LV, where the optimal detection pH was determined to be pH 5. Linear coefficients (R^2) of 0.997 and 0.999 were obtained for pH 5 and 7 as shown in Fig. 4 (c).

It should also be noted that a second peak at ~0.6 V shows minimal variation in peak current and is most likely due to the absorption of the electrolyte onto the electrode surface. This is theorised to be due to the formation of NaOH in the water/ethanol mixture and is dependent on the [NaCl aq]. It is observed in the blank electrolyte (see Fig. S2), but is not observed in NaCl aq [37]. However, this has been observed for other carbon material electrodes [38]. Further investigation into why this only occurs for within the aqueous/organic electrolyte and the exact mechanisms of this is needed. The secondary 5-MTHF peak is no longer observed at these lower concentrations. However, for this work, the peak at ~0.2 V was utilised for this analytical investigation. These calibration curves reveal distinct trendlines, highlighting the influence of pH on the electrochemical response. As shown in Fig. 4 (a), the peak potential for 5-MTHF at pH 5 shifts from 0.15 V to 0.20 V as the concentration of 5-MTHF increases. This phenomenon might be due to the dominant influence of pH at low concentrations, stabilizing the peak potential as the concentration increases.

At pH 7, the linearity of the response is less pronounced, with linear coefficients (R^2) of 0.9979 at pH 5 and 0.99691 at pH 7, as shown in Fig. 4 (b) and (c). The limit of detection (LOD) was calculated using the formula [LOD = 3.3σ/S], resulting in an LOD of 0.509 μM at pH 7 and 0.477 μM at pH 5. As expected, the LOD for biological samples is slightly higher due to interference effects, although still within the therapeutically relevant range, as shown in Table 1. While the sensor shows optimal performance at pH 5, it remains effective at pH 7, demonstrating its potential for use in diverse biological matrices.

The intra- and inter-day repeatability evaluations also yielded low relative standard deviations (RSD) with an averaged value of 7.64 % (5-MTHF) and 3.10 % (LV) for intra-day data and 5.39 % (5-MTHF) and 2.08 % (LV) for inter-day results over the concentration range investigated (See Table 2). The inter-day calculations were carried out over 5 days on 6 different electrodes illustrating the stability of this approach.

3.3. Simultaneous detection of LV and 5-MTHF

While previous research has explored the electrochemical detection of LV and the data presented in Figs. 2 - 4 illustrate that 5-MTHF can also be detected electrochemically, to exploit and optimise the potential clinically significance or impact of this approach, simultaneous detection is essential. Fig. 5 shows the SWV observed for solutions containing both LV and 5-MTHF. Based on the data presented above and previous work [17], the peak at ~0.25 V corresponds to 5-MTHF, while the peak at ~0.65 V corresponds to LV. Both peaks increase in a linear fashion with increasing concentrations as shown in Fig. 5, over the concentration range 0 ≤ [5-MTHF] ≤ 10.882 μM and 0 ≤ [LV] ≤ 10 μM. The

Table 1
Analytical results of this study.

pH	5-MTHF LOD μM	LV LOD μM	Linear Range μM
5	0.477		2.187 – 21.865
7	0.509		2.187 – 21.865
Human pooled serum	ND	10*	ND
Artificial Urine	1.72	1.664	0 – 10.88 (5-MTHF) 0 – 10 (LV)

Table 2
Analytical results of this study including mean, standard deviation (SD) and relative standard deviation (RSD) of replicates of each LV concentration and each 5-MTHF concentration (n = 9).

[5-MTHF]/ μM	Average	SD	*RSD*	[LV]/ μM	Average	SD	*RSD*
2.721	0.057	0.007	11.6	10	0.661	0.009	1.32
5.441	0.194	0.015	7.74	10	0.638	0.011	1.76
8.162	0.285	0.014	5.02	10	0.612	0.009	1.47
10.882	0.443	0.027	6.19	10	0.616	0.016	2.57
Intra-day	0.246	0.016	7.64	Intra-day	0.632	0.011	3.10
Inter-day*	0.059	0.024	5.39	Inter-day	0.656	0.013	2.08

*data over 5 days see Fig. S3, a secondary concentration is also included in Fig. S4.

values obtained were consistent with those values obtained for similar concentrations of each analyte individually in the data presented above and previously [17]. LOD for this combined detection was also in agreement and intra- and inter-day repeatability evaluations similarly yielded low values (Table 2).

3.4. Biological analysis

To evaluate the capability of our method for monitoring LV and 5-MTHF simultaneously, two biological matrices were evaluated: artificial urine and human pooled serum. These matrices were chosen to demonstrate the feasibility and practical application of our sensor for monitoring drug therapies across different biological samples commonly used by clinicians.

The impact of these matrices on selectivity and sensitivity was evaluated. Extending from previous studies that focused solely on LV [17], this work explores the capacity of this approach for simultaneous detection of LV and 5-MTHF in biological matrices. Due to limited information regarding 5-MTHF detection through electrochemical methods, our findings were compared with both electrochemical and standard chromatographic techniques [15,16].

Examining various biological matrices is critical for evaluating the developed sensor's real-world applicability and performance. To investigate this, we spiked artificial urine and human pooled serum with defined concentrations of both LV and 5-MTHF to assess the analytical performance of this approach in detecting therapeutic concentrations of LV. This comprehensive evaluation allows for validating the sensor's applicability in biological matrices and demonstrates its potential for point-of-care drug therapy monitoring. The results from spiked samples offer valuable insights into the sensor's performance under physiological conditions, highlighting its robustness and appropriateness for practical applications in personalized medicine. The absence of a requirement for prior sample preparation accentuates the sensor's ease of use, presenting a simplified process for drug monitoring. This user-friendly approach can significantly benefit both patients and healthcare providers, streamlining the drug monitoring processes.

3.4.1. Human pooled serum analysis

The primary methods for detecting LV and 5-MTHF in blood and human serum are chromatographic techniques [25,39–43]. From these, it is evident that approximately one third of the folate in serum and urine is in the form of folinic acid with the reminder found as 5-MTHF. These methods offer excellent detection limits and can be considered the "gold standard" for quantification and identification but are hindered by size, cost, complexity, sample preparation and time to results for POC applications.

From typical dosages of LV (50 to 500 mg m^{-2}), the resulting plasma concentration is usually 24 μM, with a steady state stabilising at 3.2 μM [44,45]. Previous electrochemical work has shown that it is very difficult to detect this concentration range of LV in human pooled serum. For this work, serum samples were spiked with 19.044 μM 5-MTHF and a fixed 10 μM LV concentration, and results were recorded directly on the bare screen-printed carbon electrodes (SPCEs). Fig. 6 shows a comparison of the SWV data observed for these spiked samples and the neat serum sample.

The addition of LV to a neat human pooled serum sample, saw a single peak at ~0.8 V (see Fig. 6) which was shifted compared to that observed in Fig. 4 [17,22]. Upon addition of both LV and 5-MTHF, no response was observed for 5-MTHF and a decrease in the peak associated with LV was observed. Issues encountered for serum samples, such as poor electron kinetics, may be responsible for some of the inconsistent results observed. Therefore, a dilution step was investigated. This did not show any improvement in the response, with only one peak being observed, and this peak decreasing in intensity upon addition of 5-MTHF although not in any discernible trend (Figs. S1, 5 and 6). There was also no observable linear trend. This was consistent with the complexity and viscosity of the serum which would have impacted on the mass transport through this biological matrix hindering diffusion and migration [17]. However, there is likely more complex interactions occurring within this sample matrix that results in no observed peak for 5-MTHF. Further investigation into this and its interactions with LV as well as work to improve both sensitivity and selectivity is required before this matrix could be considered for monitoring both LV and 5-MTHF, or indeed LV as 5-MTHF is likely to be present at some level.

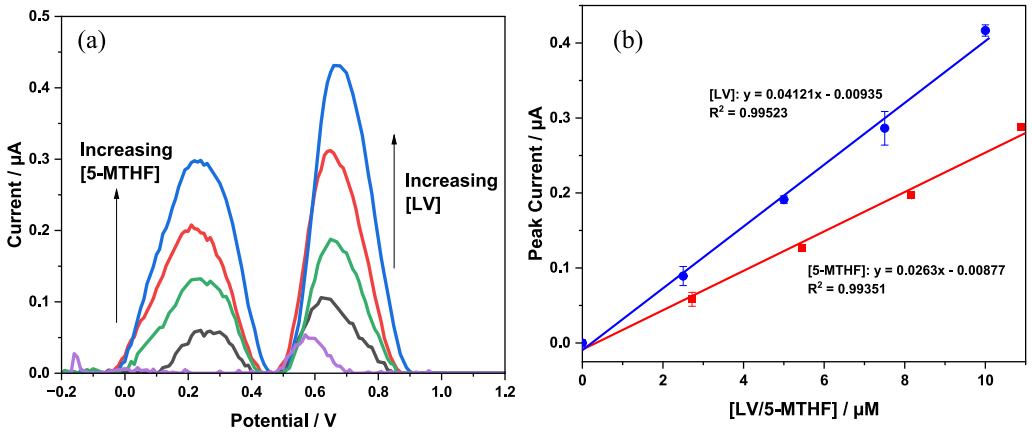

Fig. 5. (a) Typical SWV for increasing [5-MTHF] and [LV] in EtOH: 0.1 M NaCl aq (1:9 v/v) at pH 7 over the concentration range 0 ≤ [5-MTHF] ≤ 10.882 μM and 0 ≤ [LV] ≤ 10 μM scanned over the potential range 0 ≤ E≤1.2 V vs Ag at a pulse amplitude of 100 mV and frequency of 50 Hz. (b) Typical trend of maximum i_p at 0.25 V and 0.65 V at (Red squares) 5-MTHF and (Blue circles) LV for data presented in (a). Error bars represent the standard deviation obtained from triplicate results.

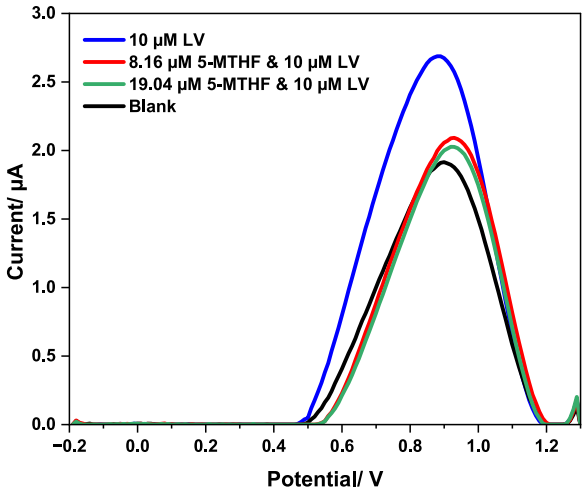

Fig. 6. Typical SWV for human pooled serum (black line), human pooled serum spiked with 10 μM LV (blue line), and human pooled serum spiked with 5-MTHF (8.16 & 19.04 μM) and fixed 10 μM LV scanned over the potential range 0 ≤ E≤1.3 V vs Ag at a pulse amplitude of 100 mV and frequency of 50 Hz.

3.4.2. *Artificial urine analysis*

LV is primarily excreted/eliminated in urine (~90 %), and mainly as the unchanged drug with a half-life of approximately 32 mins [17,46]. Therefore, this is an important matrix to assess for potential LV detection and monitoring. In addition, most of the folate eliminated in urine will be in the form of 5-MTHF, making this an ideal matrix to monitor both LV and 5-MTHF. Following the preparation of artificial urine as previously described [17], LV and 5-MTHF were spiked directly into the samples using four different concentrations of each alongside a blank control (Table 1).

The peaks at ~0.20 V and 0.65 V, corresponding to 5-MTHF and LV respectively were monitored in artificial urine. Consistent with previous electrochemical studies of analytes in artificial urine, there is a small peak observed at ~1.0 V, however, this does not interfere with the detection of either 5-MTHF or LV. The peak potential of 5-MTHF is relatively more consistent than the peak potential of LV. It can be seen from Fig. 7 that the peak potential of LV slightly shifts from 0.61 V to 0.65 V. This shift is most likely due to small variations in pH values as well as an impact due to the viscosity of the sample, which has been seen previously [47–51]. However, the shift, like previously observed shifts, is within the working parameters of the sensor and can be utilised for analytical purposes.

The % recovery ratio for LV and 5-MTHF in artificial urine is shown in Table 1. Based on these results, it is clear that this is a viable approach for POC monitoring of LV and 5-MTHF, with a typical recovery rate of 96.6 % and 97.9 % across the four different concentrations of LV and 5-MTHF which were used to spike the artificial urine. Based on these results the limit of detection (LOD) within artificial urine is 1.664 μM and 1.72 μM respectively. The LOD for LV is slightly lower than previously observed for this matrix [17,22].

To further explore the accuracy of the 5-MTHF response, artificial urine samples containing different concentrations of 5-MTHF were spiked with 10 μM LV. This is above the steady-state plasma concentration of 3.2 μM but below the typical high values usually observed at ~24 μM. The concentrations of 5-MTHF were altered over the range 8.16 to 19.04 μM [17,44,45]. The resultant peak corresponding to 5-MTHF at ~0.25 V, showed a linear dependence on [5-MTHF] with peak currents consistent with the data reported in Figs. 6, 7 and 8. This highlights the independence of the semi-quantitate data observed for 5-MTHF despite relatively high concentrations of LV. A linear coefficient (R^2) of 0.9947 is obtained for 5-MTHF. The % recovery ratio for each sample is shown in Table 3. Based on these results, it is clear that this is a viable approach for POC monitoring of 5-MTHF simultaneously with LV, with a typical recovery rate of 100.05 % across the five different concentrations of 5-MTHF which were used to spike the artificial urine. The repeatability evaluations for LV within this data also yielded low relative standard deviations (RSD) with an averaged value of 3.44 %.

4. Concluding remarks

In summary, this contribution showcases the successful application of SWV for monitoring LV and its metabolite, 5-MTHF, in urine. This represents a rapid, easy to use, cheap alternative to current chromatographic and immunoassays approaches facilitating a viable point-of-care approach for the monitoring of a therapeutic drug and its metabolite to provide clinically relevant information directly addressing the current gap for achieving personalized precision medicine. No significant interferences were observed for analysis in artificial urine, allowing for quantitative analysis of both LV and 5-MTHF at therapeutically relevant concentrations. We have shown that both LV and 5-MTHF can be detected in artificial urine and have achieved this without the requirement for complex, costly and time-consuming sample extractions procedures. This represents a strong proof-of-concept for the use of SWV for the monitoring of LV and 5-MTHF simultaneously. Further analysis is still required to facilitate the transition of this approach into the clinical arena, this would include the analysis of clinical samples and the impact of commonly consumed substances including other pharmaceutical substances. The results for human pooled serum were not as promising, with no response being observed for 5-MTHF and inconsistencies in the observation of LV making it non-viable as a potential sample matrix for the current approach. Substantial work would be needed to address this issue and provide a more selective and sensitive method for the

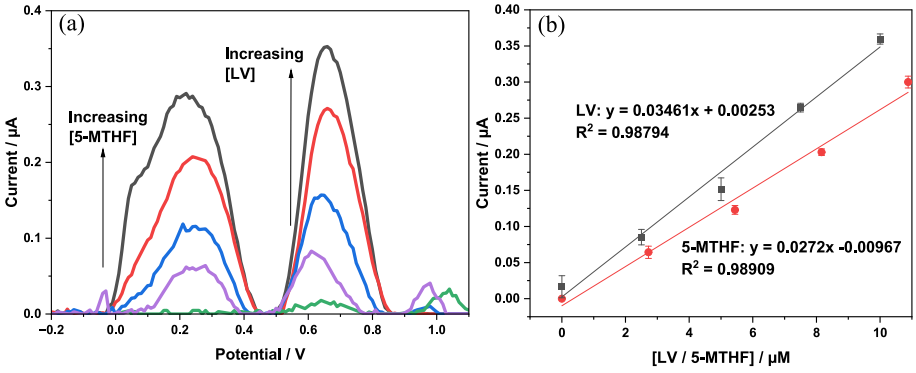

Fig. 7. (a) Typical SWV to increasing [LV] (0 ≤ [LV] ≤ 10 μM) and [5-MTHF] (0 ≤ [5-MTHF] ≤ 10.88 μM) in artificial urine at pH 7 over the potential range 0 ≤ E≤1.3 V vs Ag at a pulse amplitude of 100 mV and frequency of 50 Hz. (b) Inset shows the typical trend of maximum current at 0.25 V and 0.65 V against [5-MTHF] and [LV].

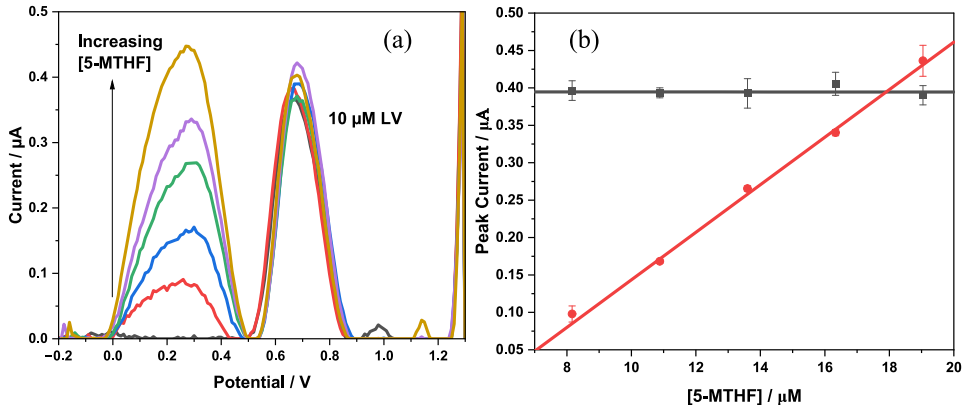

Fig. 8. (a) Typical SWV to [LV] (10 μM) and [5-MTHF] (8.16 ≤ [5-MTHF] ≤ 19.04 μM) in artificial urine at pH 7 over the potential range 0 ≤ E≤1.3 V vs Ag at a pulse amplitude of 100 mV and frequency of 50 Hz. (b) The typical trend of maximum current at 0.30 V against [5-MTHF] and current at 0.70 V for [LV].

Table 3
% recovery of [5-MTHF] spiked into Artificial Urine.

[5-MTHF] (μM)	*Recovery %*
8.16	102 ± 4.2
10.88	98 ± 0.8
13.60	101 ± 0.9
16.33	99 ± 0.8
19.04	101 ± 3.5

All values based on averaged results from triplicate independent measurements.

monitoring of both LV and 5-MTHF in human serum, or indeed direct detection in blood. Nonetheless, the approach described here shows a highly promising path for developing portable electrochemical sensors that could serve as point-of-care devices, marking a significant advancement towards the ultimate goal of personalized precision medicine.

CRediT authorship contribution statement

Pui Hang Shum: Writing – original draft, Formal analysis, Data curation. **Lynn Dennany:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

All data underpinning this publication are openly available from the University of Strathclyde Knowledge Base at https://doi.org/10.15129/cd535edf-6f15-4d12-bcd0-c212246375f4 .

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jelechem.2024.118666.

References

[1] J. Jolivet, Eur. J. Cancer 31 (1995) 1311–1315.
[2] A. Zaniboni, J. Chemother. 1 (1989) 330–337.
[3] J.S. Lee, W.K. Murphy, M.H. Shirinian, A. Pang, W.K. Hong, Cancer Chemother. Pharmacol. 28 (1991) 199–204.
[4] E. Koch, S.K. Story, L.J. Geskin, Leuk. Lymphoma 54 (2013) 2448–2451.
[5] W. Archie Bleyer, Cancer 63 (1989) 995–1007.
[6] R. Ferone in Antimalarial Drug II, Current Antimalarial and New Drug Developments 1984 Springer 207 221.
[7] F.M. Sirotnak, R.C. Donsbach, D.M. Moccio, D.M. Dorick, Cancer Res. 36 (1976) 4679–4686.
[8] J. Jolivet, G. Jansen, G.J. Peters, M.-F. Pinard, J.H. Schornagel, Biochem. Pharmacol. 47 (1994) 659–665.
[9] W. Wilmanns, H. Sauer, A. Schalhorn, in *Cancer Chemo-and Immunopharmacology: 1*, Springer, Chemopharmacology, 1980, pp. 42–49.
[10] G. Peters, C. Van der Wilt, C. Van Groeningen, K. Smid, S. Meijer, H. Pinedo, J. Clin. Oncol. 12 (1994) 2035–2042.
[11] J. Zittoun, A.P. Tonelli, J. Marquet, E. De Gialluly, C. Hancock, A. Yacobi, J. B. Johnson, Eur. J. Clin. Pharmacol. 44 (1993) 569–573.
[12] H. Taguchi, J. Nutr. Sci. Vitaminol. 27 (1981) 283–290.
[13] D. G. Priest, J. C. Schmitz, M. A. Bunni and R. K. Stuart, 1991.
[14] J. Allen, G. Rosen, H. Juergens, B. Mehta, J. Neurooncol 1 (1983) 39–44.
[15] F. Sczesny, G. Hempel, J. Boos, G. Blaschke, J. Chromatogr. B Biomed. Sci. Appl. 718 (1998) 177–185.
[16] A. Torres, S. A. Newton, B. Crompton, A. Borzutzky, E. J. Neufeld, L. Notarangelo and G. T. Berry, in *JIMD Reports, Volume 24*, eds. J. Zschocke, M. Baumgartner, E. Morava, M. Patterson, S. Rahman and V. Peters, Springer Berlin Heidelberg, Berlin, Heidelberg, 2015, DOI: 10.1007/8904_2015_445, pp. 91-96.
[17] P.H. Shum, L. Dennany, Analyst 149 (2024) 2655–2663.
[18] C. McLean, B. Tiller, R. Mansour, K. Brown, J. Windmill, L. Dennany, J Electroanal Chem 909 (2022) 116149.
[19] K. Brown, C. Hoskins, L. Dennany, Bioelectrochemistry 146 (2022) 108164.
[20] A. Ameen, K. Brown, L. Dennany, J. Electroanal. Chem. 909 (2022) 116141.
[21] A. Ameen, K. Brown, L. Dennany, Journal of the Electrochemistry Society 168 (12) (2021) 126510.
[22] K. Brown, L. Dennany, Sensor and Actuator Reports 3 (2021) 1000065.
[23] E. Rozoy, S. Simard, Y. Liu, D. Kitts, J. Lessard, L. Bazinet, Food Chem. 132 (2012) 1429–1435.
[24] M.D. Lucock, M. Green, M. Priestnall, I. Daskalakis, M.I. Levene, R. Hartley, Food Chem. 53 (1995) 329–338.
[25] M.D. Lucock, R. Hartley, R.W. Smithells, Biomed. Chromatogr. 3 (1989) 58–63.
[26] K. Hyland, R. Surtees, Pteridines 3 (1991) 149–150.
[27] J. Lankelma, E. Van Der Kleijn, M.J.T. Jansen, J. Chromatogr. B Biomed. Sci. Appl. 182 (1980) 35–45.
[28] R. Šelešovská, L. Bandžuchová, T. Navrátil, J. Chýlková, Electrochim. Acta 60 (2012) 375–383.
[29] D. Manoj, D. Ranjith Kumar, J. Santhanalakshmi, Appl. Nanosci. 2 (2012) 223–230.
[30] M. Arvand, A. Pourhabib, M. Giahi, J. Pharm. Anal. 7 (2017) 110–117.
[31] A.-C. Le Gall, C.M.G. van den Berg, Anal. Chim. Acta 282 (1993) 459–470.
[32] E.M. Materon, A. Wong, O. Fatibello-Filho, R.C. Faria, J. Electroanal. Chem. 827 (2018) 64–72.
[33] Q. Cheng, Y. Du, K. Wu, J. Chen, Y. Zhou, Colloids Surf. B Biointerfaces 84 (2011) 135–139.
[34] A. Mehrabi, M. Rahimnejad, M. Mohammadi, M. Pourali, J. Appl. Electrochem. 51 (2021) 597–606.
[35] K. Brown, C. Jacquet, J. Biscay, P. Allan, L. Dennany, Anal. Chem. 92 (2020) 2216–2223.
[36] M. Parrilla, F. Joosten, K. De Wael, Sens. Actuators B 348 (2021) 130659.
[37] L. Százdi, J. Gulyás, B. Pukánszky, Compos. A Appl. Sci. Manuf. 33 (10) (2002) 1361–1365.
[38] J.N. Barisci, G.G. Wallace, R.H. Baughman, J. Electrochem. Soc. 147 (12) (2000) 4580–4583.
[39] X.-H. Zheng, L.-Y. Jiang, L.-T. Zhao, Q.-Y. Zhang, L. Ding, J. Pharm. Anal. 5 (2015) 269–275.
[40] R.J. Pawlosky, V.P. Flanagan, C.M. Pfeiffer, Anal. Biochem. 298 (2001) 299–305.
[41] R. Kok, D. Smith, J. Dainty, J. Van Den Akker, P.M. Finglas, Y. Smulders, C. Jakobs, K. De Meer, Anal. Biochem. 326 (2004) 129–138.

[42] K. Pietrzik, L. Bailey, B. Shane, Clin. Pharmacokinet. 49 (2010) 535–548.
[43] J. Lankelma, E. Van Der Kleijn, J. Maria, J. Chromatogr. B Biomed. Sci. Appl. 182 (1980) 35–45.
[44] F. Trave, Y. Rustum, N. Petrelli, L. Herrera, A. Mittelman, C. Frank, P. Creaven, J. Clin. Oncol. 6 (1988) 1184–1191.
[45] R.L. Schilsky, M.J. Ratain, JNCI: Journal of the National Cancer Institute 82 (1990) 1411–1415.
[46] Hegde VS, Nagalli S. Leucovorin. [Updated 2023 Jul 3]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553114/.
[47] K. Brown, R.S. Blake, D.W. Robinson, L. Dennany, J. Electroanal. Chem. 941 (2023) 117521.
[48] E. Dokuzparmak, K. Brown, L. Dennany, Analyst 146 (2021) 3336–3345.
[49] K. Brown, M. McMenemy, M. Palmer, D.W. Robinson, M.J. Baker, P. Allan, L. Dennany, Anal. Chem. 91 (2019) 12369–12376.
[50] K. Brown, A.J. Stewart, L. Dennany, Anal. Chem. 90 (2018) 12944–12950.
[51] A.J. Stewart, J. Hendry, L. Dennany, Anal. Chem. 87 (23) (2015) 11847–11853.