

## Morin-modified carbon paste electrode as a promising tool for monitoring aluminium: An initial study

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Received: June 15, 2022; Accepted: June 22, 2022

*This article introduces spectrophotometric and electrochemical investigations of the complex-forming reaction between morin (MO) and the aluminium ions in mild acidic media. Already in the initial stage, the latter measurements have indicated that a carbon paste electrode bulk-modified with a small portion of MO could serve as a simple voltammetric sensor for indirect monitoring the Al(III) species in selected samples, such as some pharmaceutical products.*

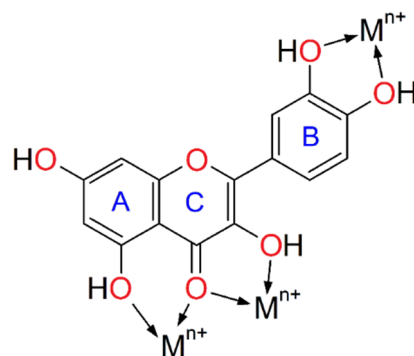
**Keywords:** Morin; Aluminium(III); Complex-forming reaction (chelation); UV-Vis spectrophotometry; Voltammetry; Carbon paste electrode.

### Introduction

It is generally known that a complexation of morin (MO; 2',3,4',5,7-penta-hydroxyflavone, C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>) with aluminium ion, Al<sup>3+</sup> (or also Al(III) species, respectively) leads to fluorogenic deeply yellow complex of the chelate type with absorption maximum at 421 nm [1,2]. According to these reports, the stoichiometric composition of the resultant complex is 2:3 (Al:MO) in mild acidic media mixed with ethanol (50%, v/v). In 1995, the reaction of morin with Al<sup>3+</sup> was used for development of a very sensitive spectrophotometric assay [3]. A more detailed study mentioned in this paper has shown that aluminium salts are able to form complexes also with other flavonoids. For instance, this is the case of quercetin and Fig. 1 illustrates all its possible chelating sites [4].

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**Fig. 1** Chelating sites of quercetin (redrawn and rearranged from [4])

The ratio of such chelating sites varies significantly in dependence of pH given by the experimental medium chosen, where the accompanying anionic ligands (hydroxyl, acetate, phosphate, etc.) may also participate in the complex-forming reaction [4,5]. Hence, it can be assumed that the complexation between MO and  $Al^{3+}$  ions is not unambiguously defined and its utilization for analytical purposes would have some limitations, especially in terms of sensitivity and selectivity.

Besides spectrophotometry, voltammetry is also applicable [6], when hitherto the only method has been developed and proposed for monitoring of aluminium in different types of water samples (namely: river, tap, and bottled). The respective approach was based on the shift of the oxidation peak of MO involved in the complex-forming reaction toward higher positive potentials ( $E_p^a$ ). The respective measurements were usually performed at unmodified glassy carbon electrode (GCE), while MO was dissolved in the working medium [6].

This article reports on the initial research combining, for the first time, spectrophotometric and voltammetric investigations of the complexation between MO and  $Al^{3+}$  ions. In addition, this work also introduces a carbon paste electrode (CPE) [7] bulk-modified with MO as a promising electroanalytical tool for monitoring of Al(III) species in selected samples, such as some pharmaceutical products, where the occurrence of metal ions capable of chelating with MO is not the case and hence, their interference do not need to be considered.

## Experimental

### Chemicals

Morin hydrate and aluminium nitrate nonahydrate were obtained from Merck KGaA (Darmstadt, Germany). Glacial acetic acid and sodium acetate for preparation of  $0.1 \text{ mol L}^{-1}$  acetate buffer (AcB; pH 4) were purchased from Lach-Ner (Neratovice, Czech Republic). Ultrapure water of resistivity  $\leq 18.3 \text{ M}\Omega \text{ cm}$  was prepared by passing of the already deionized water through a Milli-Q<sup>®</sup> purification unit (Merck Millipore, Burlington, MA; USA).

## Ultraviolet–visible spectroscopy

Absorption spectra in ultraviolet–visible region were recorded on a UV-Vis spectrophotometer UV2450 from Shimadzu (Kyoto, Japan) using 1 cm quartz cuvette (Fisher Scientific Pardubice, Czech Republic) in the range of wave-lengths from 800 to 200 nm at a scanning speed of  $0.5 \text{ nm s}^{-1}$ .

## Voltammetry and pH-measurements

Each voltammetric experiment was carried out in electrochemical glass cell containing 10 mL  $0.1 \text{ mol L}^{-1}$  AcB (pH 4) with immersed operational electrodes; namely, bare CPE or CPE/MO (working), Ag/AgCl/ $3 \text{ mol L}^{-1}$  KCl (reference), and platinum wire (auxiliary electrode). These electrodes were connected to a potentiostat/galvanostat Autolab/PGSTAT101 operated through a NOVA 1.11 software (both Metrohm Prague, Czech Republic).

Wherever needed, measurements of pH values were carried out with an InoLab portable device (pH 720 model) devised with the combined pH-glass electrode (Sen Tix-41 type) and calibrated using a set of commercial buffers (all three items WT Werke, Germany).

## Preparation of carbon paste electrode modified with morin

A portion of 0.79 g powder of natural (chemically purified) graphite with particle size of  $\geq 5 \text{ }\mu\text{m}$  (Graphite, Týn nad Vltavou, Czech Republic), 0.20 g of silicone oil (Lučební závody, Kolín, Czech Republic), and 0.01 g of morin were manually mixed altogether and then thoroughly homogenised for 15 min to obtain the proper carbon paste electrode material containing 1% MO (w/w). The resultant mixture was packed into the Teflon<sup>®</sup> piston-driven electrode holder (with active surface,  $d_{act}$ , 3 mm in diameter). The bare CPE was prepared in similar way; however, without the addition of MO.

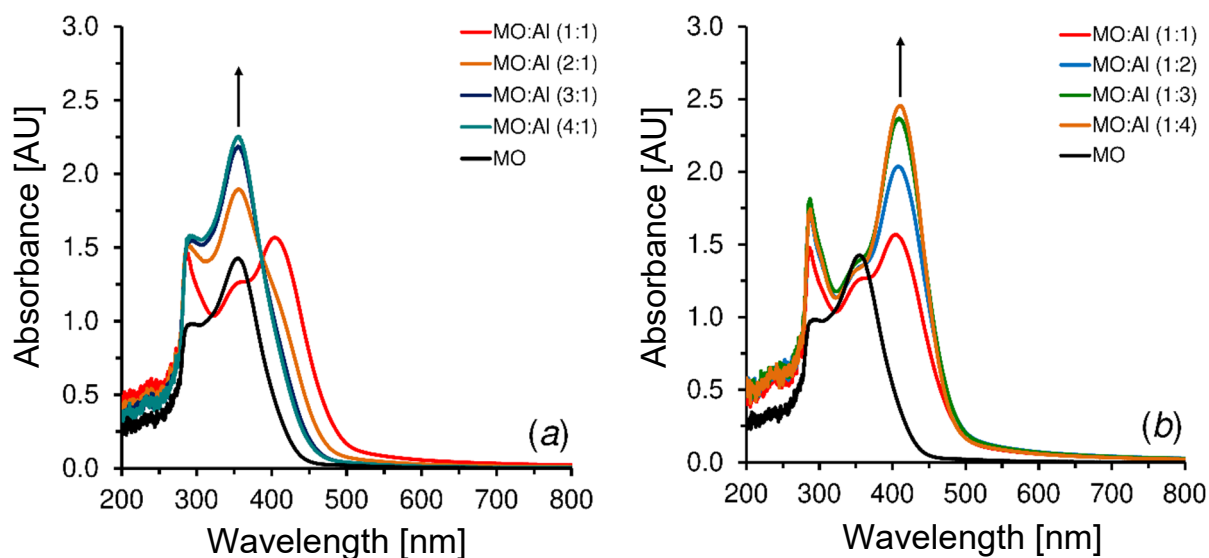
## Voltammetric measurements

Electrochemical detection of MO alone and morin complexed with Al(III) species was performed using square-wave voltammetry in  $0.1 \text{ mol L}^{-1}$  AcB (pH 4) by setting the following parameters (unless stated otherwise): potential range ( $E_v$ ) from 0.0 to +1.0 V, reaction time ( $t_{rce}$ ) of 10 min, potential amplitude ( $E_{ampl}$ ) of 25 mV, potential step ( $E_{step}$ ) of 5 mV, and frequency ( $f$ ) of 20 Hz.

## Results and discussion

### UV–Vis measurements of aluminium(III)-morin complex

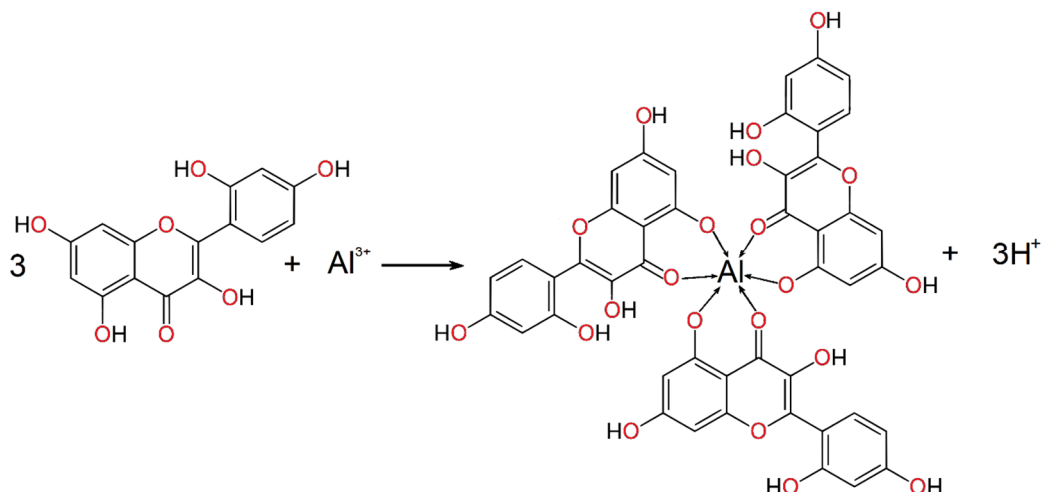
The spectrophotometry in UV-Vis region was chosen as suitable technique for definition of the complexation process *via* the reaction stoichiometry, when different concentration ratios between  $\text{Al}^{3+}$  and MO were measured after 12 min. Fig. 1. shows absorption spectra obtained for morin and aluminium(III)-morin complex. A maximum absorption at a wavelength of 356 nm was observed for morin in acetic-acetate medium, while the maximum absorption of the Al(III)-MO complex appeared at around 410 nm. As can be deduced from the comparison between Fig. 2a and Fig. 2b, the aluminium central atom can be surrounded by up to three MO molecules ([8]; see also Fig. 3) or MO can bind more than one Al(III) atoms [9]. Based on these findings, it can be predicted that a linear range of the derived analytical method for quantification of the aluminium content would strictly depend on the total morine content in non-oxidized or unpolymerized forms, because of instability of MO (as a flavonoid compound) in the contact with atmospheric oxygen [10].



**Fig. 2** UV-Vis absorption spectra of different concentration ratios between MO and  $\text{Al}^{\text{III}}$  atoms in  $0.1 \text{ mol L}^{-1}$  AcB (pH 4) recorded after 12 min

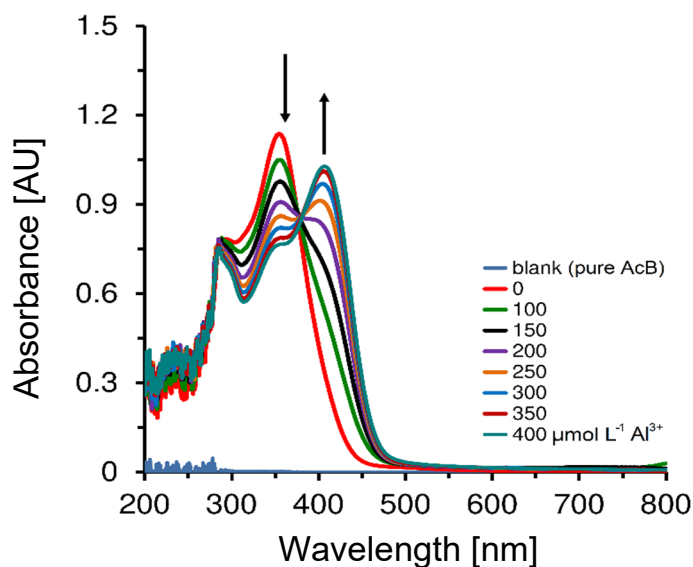
The spectrum printed in black was obtained for  $200 \mu\text{mol L}^{-1}$  MO

As shown in Fig. 4, the absorption band of MO decreases with the increasing  $\text{Al}^{3+}$  ions present in the solution, which is also reflected in the increase of the Al(III)-MO complex absorption band.



**Fig. 3** Complexation of  $\text{Al}^{3+}$  ions with MO. A scheme (redrawn and rearranged from [11])

A linear dependence between the concentration ( $c$ ) of  $\text{Al}^{3+}$  ions and absorbance ( $A$ ), at a maximum wavelength ( $\lambda_{\text{max}}$ ) of the  $\text{Al(III)}$ -MO complex at 410 nm was obtained described by eqn.  $A(\text{AU}) = 0.0024 + 0.0027c(\mu\text{mol L}^{-1})$  and characterised by the coefficient of determination of 0.9999 for concentration range of  $0\text{--}200 \mu\text{mol L}^{-1} \text{Al}^{3+}$  in the presence of  $100 \mu\text{mol L}^{-1} \text{MO}$ .



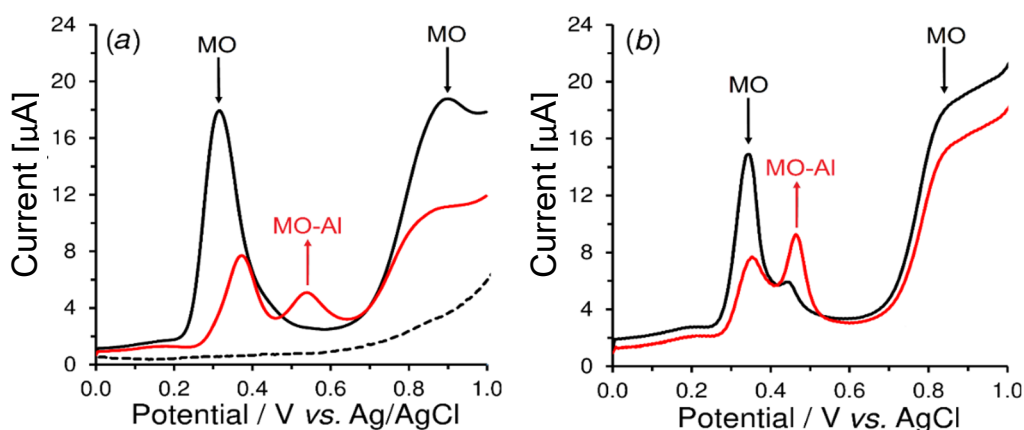
**Fig. 4** UV-Vis absorption spectra recorded for the mixtures of  $100 \mu\text{mol L}^{-1} \text{MO}$  and different content of  $\text{Al}^{3+}$  ions in  $0.1 \text{ mol L}^{-1} \text{AcB}$  (pH 4) after 12 min

#### Electrochemical behaviour of MO in presence of $\text{Al}^{3+}$ ions

As demonstrated in Fig. 5a, MO gives rise to a couple of oxidation peaks at  $+0.322$  and  $+0.906 \text{ V}$  in a mild acidic medium at the bare CPE. However, if the  $\text{Al}^{3+}$  ions are present in the working solution, a completely new oxidation peak is

observed at +0.589 V, with a related decrease in the heights of the above peak pair. This phenomenon can be attributed to a more difficult anodic oxidation of the complex-bound morin, which results in a shift toward higher potential values.

A similar electrochemical behaviour of MO can be observed at CPE/MO in pure working electrolyte, as depicted in Fig. 5b. The anodic peak at +0.423 V attributed to electrochemical oxidation of MO-Al complex attached onto electrode surface was higher than that in the previous case, which could be utilized in a development of more sensitive voltammetric methods for the determination of  $\text{Al}^{3+}$  ions. Herein, it must be noted that a self-overlapping anodic peak for MO at +0.347 V had also been observed but it could be split within optimisation of the electrochemical detection.



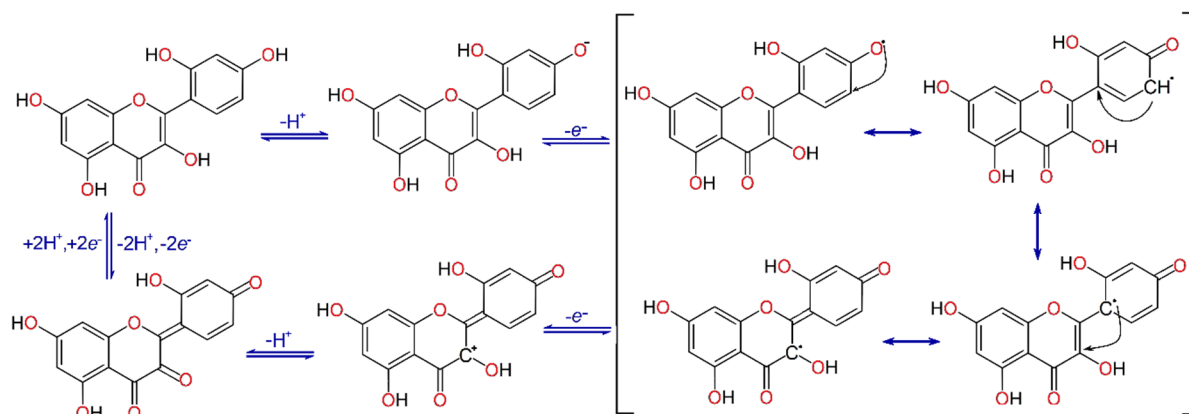
**Fig. 5** Voltammograms of 0 (dotted), 100  $\mu\text{mol L}^{-1}$  MO (black) and 100  $\mu\text{mol L}^{-1}$  MO with 400  $\mu\text{mol L}^{-1}$   $\text{Al}(\text{NO}_3)_3$  (red line) obtained at bare CPE in 0.1 mol  $\text{L}^{-1}$  AcB (pH 4) at  $t_{\text{rce}} = 90$  s,  $E_{\text{step}} = 5$  mV,  $E_{\text{ampl}} = 25$  mV, and  $f = 20$  Hz (a)

Voltammograms of blank supporting electrolyte (black) and 100  $\mu\text{mol L}^{-1}$   $\text{Al}(\text{NO}_3)_3$  (red line) obtained at CPE/MO at the above-mentioned SWV parameters (b)

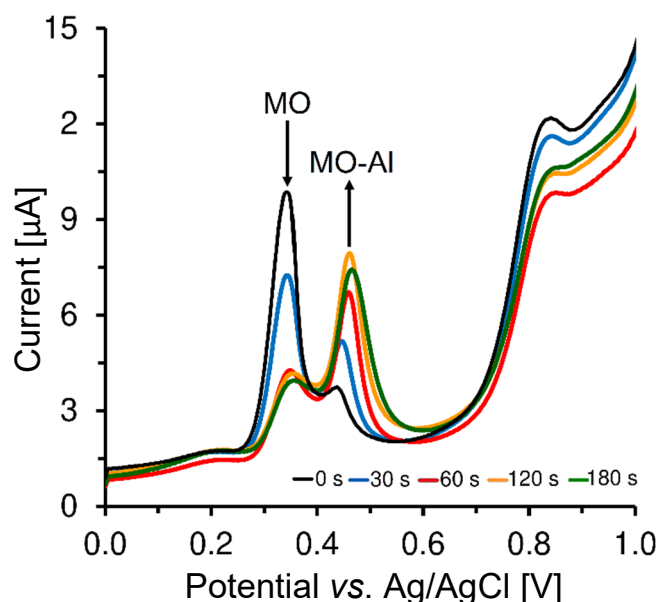
The electrochemical behaviour of MO at carbon-based electrodes in aqueous media has already been studied in order to design a mechanism of the corresponding electrode reaction [12,13]. If possible chemical side-reactions are neglected, the electrode oxidation pathway for MO will be primarily described by the reaction mechanism schematised in Fig. 6. In the individual sequences, it is seen that MO is reversibly oxidised to form *p*-quinone methide [14,15] with participation of  $2e^-$  and of  $2\text{H}^+$ .

At first glance, it is clear from the comparison of the voltammograms in Fig. 7 that the accumulation (or attachment) of  $\text{Al}^{3+}$  ions within the complexation reaction with the MO molecule is a time-dependent process.

Hence, it can be assumed that this finding on the reaction-time dependence would play an important role in optimisation of the proper composition of the electrode material, if one means the configuration of a CPE with MO added as a solid. And the amount of MO would certainly be a crucial factor to achieve the desired analytical performance, e.g., in pharmaceutical analysis.



**Fig. 6** The sequences of the electrode oxidation of MO with all the individual mesomeric structures. The overall scheme.



**Fig. 7** Set of voltammograms for measurements of  $100 \mu\text{mol L}^{-1} \text{Al}(\text{NO}_3)_3$  obtained at bare CPE/MO in  $0.1 \text{ mol L}^{-1} \text{AcB}$  (pH 4) at different reaction times (legend),  $E_{\text{step}} = 5 \text{ mV}$ ,  $E_{\text{ampl}} = 25 \text{ mV}$ , and  $f = 20 \text{ Hz}$ .

## Conclusions

The article described for the first time the combined use of UV/Vis spectrophotometry and voltammetry chosen to investigate some aspects of the complex-forming reaction between morin and aluminium ions. (*Note: This statement applies despite the already mentioned paper by Domínguez-Renedo et al [6], where both techniques had also been used, but in a limited scope only and within a wider portfolio of other instrumental measurements.*)

Based on some results of more-or-less basic study on complexation of Al(III) with morin, a carbon paste electrode bulk-modified with MO was tested to utilise this reaction for analytical purposes – for indirect determination of Al(III). It has been found that such configuration containing even a very small amount of MO admixed into the carbon paste can offer a sensor with promising performance for monitoring the content of aluminium in pharmaceutical formulations in mild acidic media. These products represent samples with specific matrix, where other metal ions being capable of chelating with MO occur only seldom if at all.

Regarding further development of the method with MO-modified CPE and its extension to other types of samples, primary attention should be paid to possible interferences from other metal ions. It is known that morin, as traditional analytical reagent [16,17], is capable of complexing numerous metal ions. Under conditions adjusted for binding  $\text{Al}^{3+}$ , which is mainly pH, the following cations are chelated, too:  $\text{Be}^{2+}$ ,  $\text{Ga}^{3+}$ ,  $\text{Sc}^{3+}$ ,  $\text{Y}^{3+}$ ,  $\text{La}^{3+}$ ,  $\text{Zr}^{4+}$ ,  $\text{Th}^{4+}$ , and  $\text{UO}_2^{2+}$  [16,17]. However, their occurrence in pharmaceutical samples is highly improbable and therefore, their interferences do not need to be assumed. At higher pHs, there are other cations that can be complexed with MO:  $\text{Cu}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$  (see e.g. [16,18,19]). Some of them are very common species accompanying the  $\text{Al}^{3+}$  ions also in pharmaceutical and clinical samples [20]; usually, at a very large excess in concentration, when they may interfere also in media with lower pH. In other words, especially  $\text{Fe}^{3+}$ ,  $\text{Ca}^{2+}$ , and  $\text{Zn}^{2+}$  represent true interfering species with respect to which the method with MO-modified CPE shall be optimised yet.

## Acknowledgement

*A support received from the Faculty of Chemical Technology at the University of Pardubice (project No. SGS-2022-002) is gratefully acknowledged.*

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