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DOI: 10.2478/cdem-2024-0005 CHEM DIDACT ECOL METROL. 2024;29(1-2):65-83

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MICROWAVE-AIDED NITRATION OF PHENOL WITH INORGANIC NITRATES: INQUIRY-BASED LEARNING EXPERIMENTS

Abstract: Current trends in organic synthesis as well as in chemistry education highlight the principles of Green chemistry and call for new synthetic procedures that conform to the actual ecological and economic requirements according to the Green deal and other global challenges. This includes, for instance, replacement of aggressive and toxic reagents, optimisation of synthetic protocols to achieve the highest possible yields within the shortest reaction times, lowering the reaction temperature, solvent recycling and waste minimisation. Considering the present technological advances, replacement of classical heating by microwave irradiation turns out to be an important tool of Green chemistry that permits significant reduction of the reaction time and increase of the yield under certain conditions. In chemistry education alike, several teaching experiments have also been reported in the latest literature to show advantages of the microwave-aided synthesis of various organic compounds or to elucidate basic chemical reactivity principles (e.g. direct carboxamide synthesis, aromatic sulphonation with rearrangement). In the present study, we design a Green chemistry education project focused on microwave-aided nitration of phenol by a set of inorganic metal nitrates (i.e. sodium nitrate, calcium nitrate, copper(II) nitrate, iron(III) nitrate) in concentrated acetic acid. These inquiry-based learning experiments proceed very easily, and the reaction conditions can be controlled to achieve the first, the second or even the third degree of nitration. Along with a necessary minimum of chemistry knowledge and skills, the proposed educational experiments on microwave-aided synthesis encourage students to explore the influence of the inorganic nitrate structure on the phenol nitration products. The basic relationship between the properties of reactants and the course of this interesting organic reaction can be easily monitored by universal pH papers and thin-layer chromatography, and subsequently explained through inductive reasoning. As such, these student-centred experiments are suitable for implementation in inquiry-based chemistry education at universities or high schools oriented in natural sciences.

Keywords: inquiry-based learning, aromatic nitration, microwave-aided synthesis, phenol, metal nitrates

Introduction

The strategy in the field of educational experiments has significantly changed in recent years due to many causes. Incentive for such innovations may stem from the turbulent development in the field of information technologies on the one hand and from advances in the methodology of laboratory work and instrumentation on the other. Undoubtedly, information technologies have entered vigorously into the realm educational chemical

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experiments (i.e. reactions whose course is monitored or driven by a computer) and further educational revolution can be expected in relation to the unprecedented development of artificial intelligence [1, 2]. The situation is similar in the area of innovative laboratory equipment such as ultrasonic and microwave reactors, or automatic chemical synthesisers that has demonstrated the powers of current material engineering, miniaturised electromechanics or so-called smart devices [3-5]. From this broad context of the state-of-the-art technologies, we would like to place special emphasis on a very small fraction, which is represented by commonly used devices like kitchen microwave ovens that can be effectively used in educational experiments [6-8].

Microwave ovens can be used in many ways for teaching purposes, especially in the area of physics and chemistry [9, 10]. An example is the demonstration of microwave heating of polar and non-polar substances [11]. Polar substances are heated by microwaves, non-polar substances are not. Probably the most appropriate use of microwave ovens in chemical experiments is the microwave heating of the reaction mixture [12-14]. It is well-known that in this way it is possible to achieve a very high yield of certain chemical reactions, and implement several complex organic syntheses within relative short time, which are difficult to accomplish by traditional synthetic protocols [15-17]. A typical reaction showcasing successful utilisation of microwave heating is the synthesis of amides [18, 19]. Heating a mixture of a carboxylic acid with an amine using a classical heating mantle or sand bath usually does not allow the required amide to be prepared in sufficient quantities, only after a relatively long reaction time such type of organic synthesis can be employed. In contrary, microwave heating permits to get the appropriate amide in a considerably shorter time in a high yield [20, 21].

This publication is, however, oriented to a slightly different area, namely to microwave-aided nitration of phenol by means of four metal nitrates (i.e. sodium nitrate, calcium nitrate, copper(II) nitrate, iron(III) nitrate) in the presence of concentrated acetic acid. Classically, sub-optimised nitration of phenol with nitric acid affords a mixed reaction product that is typically contaminated by numerous degradation and side products [22, 23]. Nonetheless, electrophilic aromatic substitutions (S_EAr) like phenol nitration belong to crucial chemical procedures and utilisation of microwave heating not only improves these reactions significantly, but renders them to various impressive educational applications [24, 25]. Equipped with the technique of microwave-aided organic synthesis, teachers may take courage to enhance their chemistry teaching efforts with inquiry-based learning approaches to support critical thinking, problem-solving skills, and, especially, the natural inclination in learners to seek answers trough research, experiments and discussion [26].

In principle, our intention is to translate the above-mentioned organic synthesis methods for application in the field of teaching and draw attention to the advantages of such procedures. In this spirit, we have tried to optimise the reaction conditions to help chemistry learners see basic rules that control the course as well as regioselectivity of phenol nitration. This study continues in our previous projects focused on implementation of microwave-aided synthesis in inquiry-based learning (e.g. synthesis of sulphanilic acid or *N*-phenylformamides) [27, 28]. Application of these experiments could be convenient at universities training future chemistry teachers and at secondary schools of a general educational nature. Accordingly, the presented study may be taken as a task template for further didactical extensions, enriching the current library of inquiry-based methods for chemistry education, which is still limited.

Theoretical framework and principles

Electrophilic aromatic substitution (S_EAr) is one of the most important reactions of aromatic hydrocarbons. The electrophilic particle (E^+) attacks the aromatic ring and replaces the hydrogen atom, as shown in the diagram (Fig. 1) [29, 30].

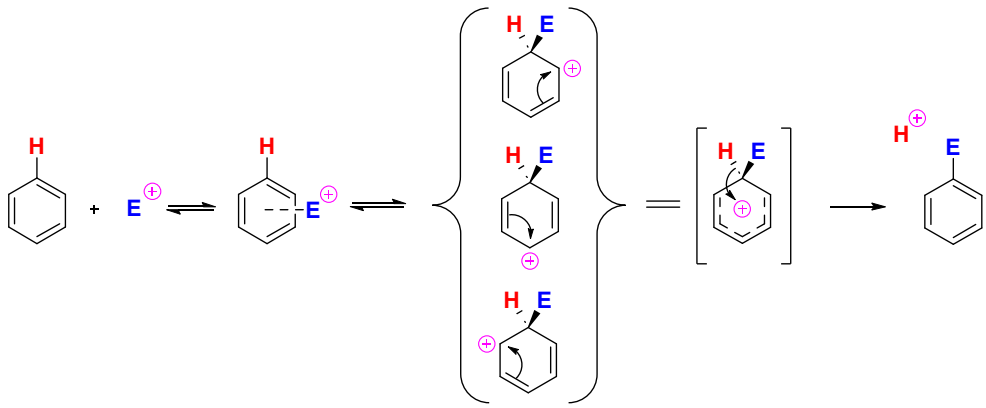

Fig. 1. Mechanism of electrophilic aromatic substitution (S_EAr)

With a suitable choice of reagents and reaction conditions in S_EAr, it is possible to introduce various substituents into the aromatic skeleton. Depending on the selected conditions and reagents, aromatic hydrocarbons may undergo nitration (the substituent is the nitro group $–NO_2$), halogenation (the substituent is a halogen, for example –Cl or –Br), sulphonation (the substituent is the sulphogroup $–SO_3H$), alkylation (the substituent is the alkyl group –R), acylation (the substituent is the acyl group –COR) and other reactions. This broad spectrum of S_EAr enables to prepare a wide number of differently substituted aromatic hydrocarbons [31, 32].

Nitration of phenols is a concrete example of electrophilic aromatic substitution, which has gained popularity over the ages. However, the course of this reaction differs significantly from the nitration of benzene in some respects. Nitration of phenols is strongly influenced by the structure of the substrate as well as the nitration reagent. Importantly, the phenol molecule contains a hydroxyl group attached to the benzene skeleton with delocalised system of π-electrons that is known to exhibit a positive mesomeric effect (*+M*) and a negative induction effect (*-I*) at the same time. Since the *+M* mesomeric effect predominates over the *-I* induction effect, the hydroxyl group acts as an electron donor on the benzene skeleton. The electron-donating action of the hydroxyl group is related to the involvement of the oxygen atom's lone electron pair in conjugation with the π-electrons of the benzene skeleton. In terms of regioselective control, electrophilic aromatic substitution in the phenol molecule is directed predominantly to the *ortho* and *para* positions, which are characterised by the highest electron density on the benzene ring. In these positions, a quantum-chemically calculated local ionisation potential, employing advanced MP2/6-311+G** method, suggests relatively increased capacity to provide electron density for new chemical bonds (Fig. 2).

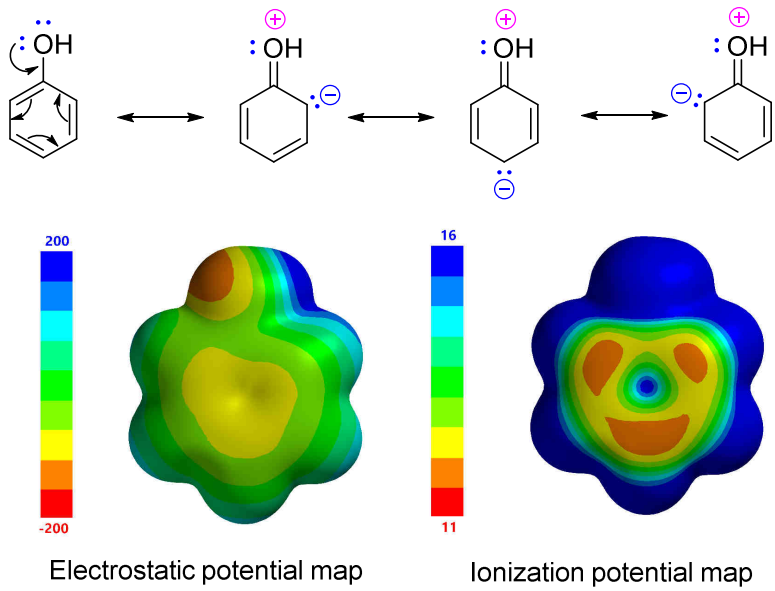

Fig. 2. Mesomeric (+*M*) effect of the hydroxyl group in phenol, electrostatic potential (ESP, in kJ/mol) and ionisation potential (IP, in eV) maps of phenol in vacuum determined with MP2/6-311+G** quantum-chemical calculation method (Source: authors)

Given there are several possibilities of replacing the hydrogen atoms of the benzene ring with a nitro group, it is possible to distinguish nitration of the first, the second and the third degree [33]. During the first-degree nitration, two products are formed: 2-nitrophenol and 4-nitrophenol. During nitration of the second-degree, two products are formed again: 2,4-dinitrophenol and 2,6-dinitrophenol. The product of the most demanding third-degree nitration is a well-known explosive 2,4,6-trinitrophenol (picric acid) (Fig. 3).

Mononitration and dinitration can be performed directly by treating phenol with nitric acid. However, introducing nitro groups within electrophilic aromatic substitution deactivates the aromatic skeleton to such extent that 2,4,6-trinitrophenol (i.e. picric acid) must be prepared by an indirect method. In practice, trinitration of phenol is achieved, for instance, through phenol sulphonation and subsequent nitration of the resulting phenol-2,4-disulphonic acid with nitric acid.

Thus, let us raise some questions concerning the topic: what is the overall effect of the hydroxyl group on the aromatic skeleton in relation to nitration? The investigation can be transformed into evaluation of two basic hypotheses:

1. The hydroxyl group facilitates the course of the reaction with its electron-donating effect
2. The hydroxyl group directs the course of nitration to *ortho* and *para* positions in accordance with the substitution effect.

Although the above-mentioned sentences have been investigated many times throughout history, let us take them as a topic for inquiry-based learning now. The influence of the hydroxyl group on the course of nitration of phenols can be estimated

from observation of two chemical reactions on so-called macroscopic level of representation [34]. The first one is: when nitrating benzene in a chemical laboratory, it is necessary to use the nitration mixture, which is a solution of concentrated nitric acid in concentrated sulphuric acid. Sulphuric acid protonates nitric acid and thus aids in the generation of the key nitronium cation (NO_2^+). The second clue is: in the case of nitration of phenols, it is sufficient to use only nitric acid as a nitration agent, which is consistent with the fact that nitronium cation can also be formed by auto-protonation and heterolytic cleavage of nitric acid itself. Trusting in such observations, the effect of the hydroxyl group can be easily discovered.

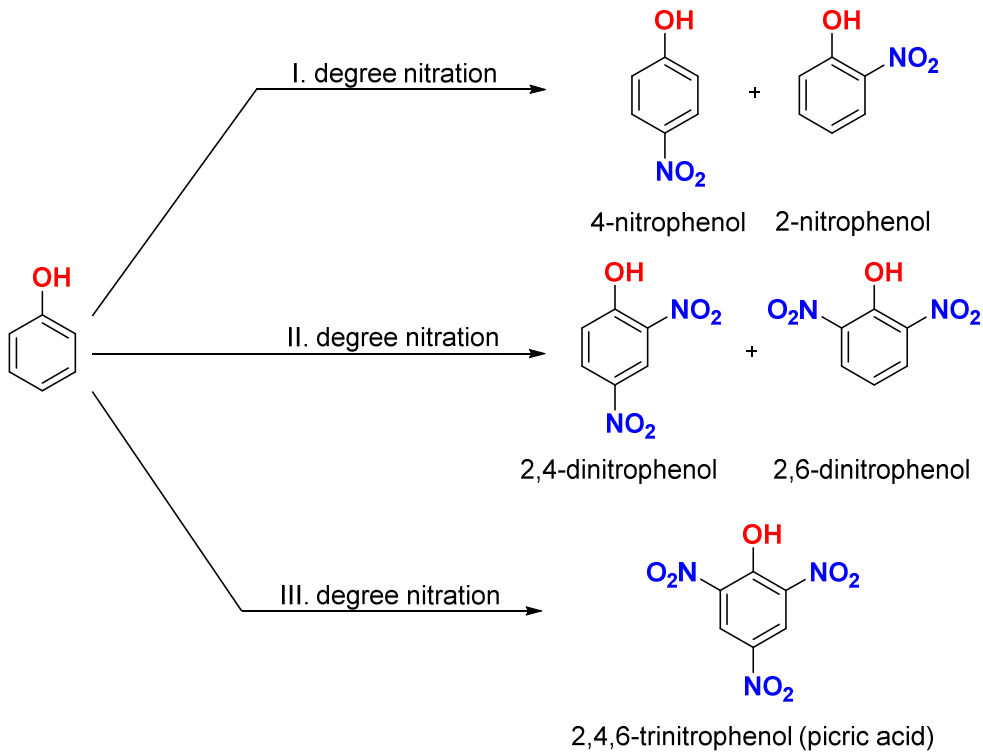

Fig. 3. Diagram of phenol nitration with the major products

Importantly, other compounds such as dinitrogen pentoxide (N_2O_5), nitrogen dioxide or dinitrogen tetroxide (NO_2, N_2O_4) can also be used for nitration [35]. In general, nitration can be carried out with a mixture of sodium nitrate or potassium nitrate in sulphuric acid [36, 37]. This nitrate-based method is one of the traditional procedures comparable to direct nitration with nitric acid [38, 39]. A somewhat different way is the use of copper(II) nitrate, bismuth(III) nitrate, ammonium cerium(IV) nitrate in the environment of acetic acid, acetic anhydride or sodium bicarbonate, which very likely involve catalytic transfer of the nitro function [40-42]. A simplified scheme of a very attractive phenol nitration by copper(II) nitrate in acetic acid or acetic anhydride is illustrated in Figure 4.

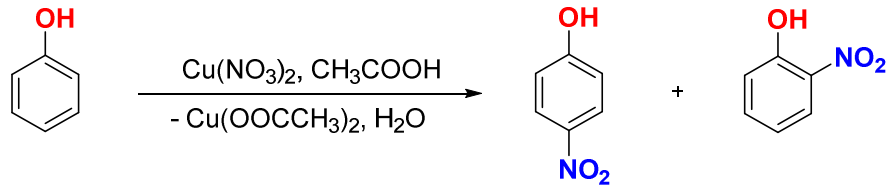

Fig. 4. Nitration of phenol with copper(II) nitrate in concentrated acetic acid, providing two main phenol mononitrates

This copper-promoted organic functionalisation can be used for nitration of aromatic hydrocarbons, which are substituted with electron-donating substituents facilitating S_EAr [43-45]. Very important role in this Menke nitration is plaid by the acetyl nitrate, which is probably formed *in situ* and may accelerate the nitration rate either *via* internally assisted deprotonation of the substate or by releasing the highly reactive nitronium cation [46-49]. Other methods of nitration are represented by the reaction of the substrate with inorganic nitronium salts (e.g. nitronium-hexafluorophosphate or nitronium-tetrafluoroborate) [50, 51].

Nitration with heavy metal nitrates is often carried out under specific conditions, for example, using heterogenous reaction systems with the nitrate fixed on sorbents like montmorillonite [52]. Such carrier substrates are, for example, Claycop, whose basis is copper(II) nitrate, or Clayfen, containing iron(III) nitrate [24, 53-55]. Another approach to shorten the reaction time, increase the yield, enhance work safety and reduce the economic costs is the application of microwaves without the use of a solvent. A concrete example can be the nitration of salicylic acid (2-hydroxybenzoic acid) with calcium nitrate in the presence of acetic acid, as a solvent-free reaction in the presence of microwave irradiation [24]. The product of this nitration is 2-hydroxy-5-nitrobenzoic acid.

The main focus of this study is the nitration of phenol with copper(II) nitrate and other metal nitrates in the presence of microwaves under solvent-free conditions. Classical nitration of phenol is a very popular reaction that is often exploited in practical exercises in organic chemistry courses, especially at universities [56, 57]. These basic organic reactions are also occasionally realised in organic chemistry exercises at secondary schools oriented at chemical technologies or in secondary vocational schools of similar focus [58-60]. In the teaching of organic chemistry at the Czech grammar schools, the experimental performance of nitration is practically not integrated into the curriculum.

As far as the chosen chemical theme is concerned, nitration of phenol is the predominantly used case in chemistry education in the Czech Republic. Less commonly, nitration of substituted phenols, such as *ortho*, *meta* and *para* cresols, or other derivatives like halogen phenols, is utilised mostly by chemistry teachers with a strong motivation for chemical experiments. On the other hand, nitration of 1-naphthol or 2-naphthol has been completely omitted in laboratory exercises at secondary schools or universities in the Czech Republic. The reason stems from the fact that nitration of naphthol isomers is associated with the formation of many different products. Such a reaction is not very transparent and turns out to be inconvenient for chemistry education. Another important question hindering the direct application of these compounds in schools is their increased toxicity and suspicion of causing cancer.

The frequent use of nitration of phenols in laboratory exercises in organic chemistry is related to a few circumstances:

1. The advantage of this reaction is its easy course, which is related to the electro-donating character of the -OH group.
2. Another advantage is the formation of clearly defined products. During nitration of the first degree, 2-nitrophenol and 4-nitrophenol are formed, 2,4-dinitrophenol and 2,6-dinitrophenol are produced in the second degree of nitration. The third-degree nitration yielding 2,4,6-trinitrophenol does not take place under standard conditions. Nitration is therefore a highly regioselective reaction, which provides products with the nitro function attached in *ortho* and *para* positions, in accordance with the resonance theory.
3. The reaction is associated with a straightforward possibility of monitoring its course using thin-layer chromatography (TLC). With silica gel and toluene as the stationary and mobile phases in TLC, respectively, the individual products can be easily detected and distinguished due to their different lipophilicity and retardation factors.
4. Separation of 2-nitrophenol and 4-nitrophenol is another important theme, which may open the way for introduction of various physical-chemical processes applied in chemical industry. The separation of the nitration products can be carried out by steam stripping, which is based on the fact that 2-nitrophenol volatiles with water vapour, while 4-nitrophenol does not exhibit this property.
5. Didactic motivation for learning this chemical topic may be also based on the practical usage of nitrophenols, since 2,4-dinitrophenol is a regular ingredient in various types of pesticides and 2,4,6-trinitrophenol (picric acid) is a notorious explosive.

Complexity of the phenol nitration, including separation of the reaction mixture, makes this reaction an attractive explanatory model that appears very often in textbooks or scripts intended for laboratory exercises in organic chemistry at universities or secondary schools in the Czech Republic as well as abroad. To illustrate this, the reader, who is familiar with Czech textbooks of organic chemistry, is kindly referred to the works by Rudolf Lukes, Otto Wichterle, Frantisek Petru, Milos Hudlicky (Zaklady preparativni organicke chemie [Fundamentals of Preparative Organic Chemistry], 1956), Miroslav Vecera, Josef Panchartek (Laboratorní prirucka organicke chemie [Laboratory Handbook of Organic Chemistry], 1987), Eugen Buchar, Josef Halbych, Jiri Borovicka (Prakticka cviceni z organicke chemie pro pedagogicke fakulty [Practical Exercises in Organic Chemistry for Faculties of Education], 1970), or Jan Wegiel, Jan Sojdr (Laboratorni cviceni z organicke chemie [Laboratory Exercises in Organic Chemistry], 1969).

For teaching purposes, this experiment may take a number of forms according to various criteria, such as:

1. Execution of the reaction at the macro, semi-micro and micro scales.
2. Application of different types of nitration agents (nitric acid, combination of sodium nitrate and sulphuric acid, etc.).
3. Execution of the reaction under special conditions (e.g. reaction in the presence of ultrasound, reaction in the presence of microwaves, etc.).

Microscale nitration of phenols can be also performed on a thin layer used for TLC. The procedure consists of the following steps:

1. A drop of phenol solution in toluene is deposited near the bottom part of a TLC plate.
2. Analogically, the phenol spot on the TLC plate is coated with a drop of nitric acid.

3. After a couple of minutes, the TLC plate is dried and inserted into a chromatographic chamber with toluene as the mobile phase.
4. The TLC plate is chemically developed by ammonia vapours and nitrous gases in a fume hood. The nitrophenols' spots are coloured yellow while the remaining phenol gives a grey-green spot.

An even simpler way of nitration on a TLC plate can be based on chemical reaction of the substrate with nitrogen oxides. For instance, a small amount of phenol can be applied to a TLC plate, which is then exposed to nitrous gases obtained by the decomposition of alkaline nitrites with mineral acids. Subsequently, the TLC chromatogram is developed and detected in a usual way [61].

Nitration of phenol can be carried out using different types of nitrating agents. Concentrated nitric acid, which has already been mentioned, can be replaced by mixtures of sodium or potassium nitrate with sulphuric acid to provide very similar products. Another possibility is the use of nitrogen oxides and organic nitrates. Each of the mentioned procedures has its advantages and disadvantages. Nitric acid has become a standard nitrating agent, which has found a broad area of applications. A mixture of alkaline nitrate with sulphuric acid allows the release of an adequate amount of nitric acid from the nitration mixture. The use of nitrogen oxides leads to a wider range of products. Organic nitrates are utilised for specific cases, nitration then takes place in a homogeneous solution and not at a phase interface. The use of nitrogen oxides as nitrating agents is, as already mentioned, suitable when the nitration is carried out on a TLC plate.

A vast majority of the above-mentioned reactions, which are traditionally carried out in a solution, can also be realised as solvent-free reactions, reactions supported with ultrasound or as microwave-aided (or microwave-assisted) reactions. These innovative modifications are mostly aimed at increasing the yield of the reaction and shortening the reaction time, which is related to the savings of energy, chemicals, etc. However, it requires special laboratory equipment such as an ultrasonic or a microwave reactor. In chemistry education, this professional laboratory instrumentation can be often replaced by more affordable tools like an ultrasonic cleaner or a kitchen microwave oven.

From the above short overview, it follows that teachers of organic chemistry have a wide range of possible approaches to elucidate the selected topics for their learners. The spectrum of specific didactic tools for teaching organic chemistry involves classical nitration of phenols by nitric acid at ambient temperature as well as modernised microwave-aided synthesis with transition metal nitrates under solvent-free conditions. Micro-scale reactions, which have gained popularity in chemistry teaching, enable to perform these motivating chemical experiments within the time limits of short study lessons using small amounts of chemicals. From TLC analyses, it is possible to obtain the key information about the product formation. Although we cannot determine the yield of the reaction using TLC, it is still possible to estimate semi-quantitatively the proportion of the products and the non-reacted starting substances in the reaction mixture.

Nitration of phenols carried out by traditional methods like the reaction of phenol with nitric acid, with a combination of alkaline nitrate and sulphuric acid, with nitrogen oxides (nitrous gases) or organic agents (e.g. benzoyl nitrate) do not require intensive heating of the reaction mixture, they take place spontaneously even under relatively mild reaction conditions [62]. A somewhat different situation arises when various nitrates are used for nitration in the presence of acetic acid or acetic anhydride because these reactions require more intensive heating. When it comes to heating the reaction mixture, the classic method

of heating, for example, on a sand bath, can be replaced by microwave heating. This method of heating the reaction mixture is very efficient and quickly leads to the desired chemical products. However, microwave heating requires certain physical-chemical properties of the substances to be met to allow its use. The basic condition is the absorption of microwave radiation by the reaction mixture. This condition is fulfilled if the reactants or intermediates belong to polar substances. If we consider the structure of the substances utilised in this study, we can state that the conditions are met and phenol as well as its nitro derivates can be heated by microwave radiation. Phenols are polar substances, the same can be said about other components of the reaction mixture (e.g. metal nitrates, acetic acid, acetic anhydride) Because the conditions of microwave heating are met in this case, the actual course of the reaction depends on other circumstances of the microwave heating, which are: microwave power, heating time of the reaction mixture, positioning of the reaction vessel in the microwave reactor. By optimising the reaction conditions, it is possible to achieve significantly better results in comparison with classical heating. For instance, microwave heating has been successfully tested on a number of reactions like hydrolysis of benzyl chloride within 3 minutes with a yield of 97 % (traditional heating takes about 35 minutes to afford the same yield), oxidation of toluene with potassium permanganate at a yield of 40 % within 5 minutes (classical refluxing of the reaction mixture takes about 1-2 hours to achieve the same yield) or esterification of benzoic acid and propan-1-ol in the presence of sulphuric acid to form propyl benzoate after 6 minutes. The given examples of chemical reactions demonstrate the efficiency of microwave heating, which affords the desired products in a sufficiently large yield within a relatively short time.

Therefore, microwave heating can be taken as an effective tool of modern chemistry methodology, which is referred to as Green chemistry. In principle, it is a laboratory and industrial philosophy that considers numerous ecological and economic aspects. The environmental aspects include, for example, the use of safe substances and the exclusion of dangerous chemical reactions. The economic aspects prompt to fully utilise the potential of chemical substances to save the material costs and energy. An ecologically conceived chemical experiment should take into account twelve principles of the so-called Green chemistry [63].

Microwave-aided chemical reactions can be carried out in a solution or in a solvent-free phase. As for the reactions carried out in solutions, it is necessary to select a polar solvent to enable the absorption of microwaves and consequent heating of the reaction mixture. If the solvent is non-polar (for example, hexane, carbon tetrachloride), microwave heating is not successful. If water or ethanol is used, microwave heating can be carried out. However, the use of toxic or non-biodegradable solvents to carry out chemical reactions is not aligned with current principles of Green chemistry.

Regarding the above statement, the implementation of chemical reactions without solvents is preferred within the framework of Green chemistry. This trend has been broadly accepted in organic synthesis for a long time and has resulted in a methodology of organic synthesis referred to as organic reactions carried out in the presence of microwaves without the use of solvents (e.g. solvent-free microwave-assisted or microwave-aided synthesis). This methodology was also adopted by us in the relevant education experiments [27]. Regarding the nitration of phenols as a solvent-free reaction carried out in the presence of microwaves, this procedure has proven to be suitable for successful performance of nitration affording the desired types of nitrophenols.

Nitration of phenols in the presence of microwaves as an inquiry-based learning experiment has currently gained much attention in connection with the trends of so-called "Green phenol experiments" [64-66]. Accordingly, we have selected the microwave-aided nitration of phenol, which was also examined by Yadav et al. [25], to extend its application towards inquiry-based learning trough comparison the effect of four inorganic nitrates (i.e. sodium nitrate, calcium nitrate, copper(II) nitrate, iron(III) nitrate) on the course of the phenol nitration. In our preliminary study with 14 subjects (12 females, 2 males), tests of the post-experimental Intrinsic Motivation Inventory with normalised *M* scores (i.e. using modified Likert scales with seven close-ended questions) suggested that a straightforward nitration of phenol by nitrous gases on a TLC plate with subsequent chromatographic analysis was perceived by the respondents as rather pleasant ($M = 0.25 \pm 0.38$), important ($M = 0.11 \pm 0.43$), not-stressful ($M = 0.13 \pm 0.47$), skilful ($M = 0.08 \pm 0.44$) and useful ($M = 0.28 \pm 0.40$) learning activity [67, 68]. Given the wide range of possible alternatives concerning both nitrate selection and replacement of acetic acid by anhydride, the study offers a wide range of possibilities for teachers to design their own microwave-aided experiments suitable for the inquiry-based learning approach.

Experimental part

Chemicals

The designed educational experiments were performed with a set of chemical compounds, which can be easily obtained on the current market from companies selling ordinary laboratory chemicals (Table 1). It is recommended to use chemicals with the purity at least of "reagent" or "purrum" grade (i.e. ≥ 95 %).

Table 1

List of used chemicals and agents

Compound (purity grade)	Supplier	Country of origin
Glacial acetic acid (p)	Lachema	Czech Republic
Phenol (p)	Lachema	Czech Republic
Toluene (p)	Penta	Czech Republic
Hydrochloric acid (36 %, p)	Analytika	Czech Republic
Aqueous ammonia (25 %, p)	Penta	Czech Republic
Sodium nitrate (p)	Lachema	Czech Republic
Calcium nitrate tetrahydrate (p)	Lachema	Czech Republic
Copper(II) nitrate trihydrate (p)	Lachema	Czech Republic
Iron(III) nitrate nonahydrate (p)	Lachema	Czech Republic
Sodium nitrite (p)	Penta	Czech Republic
2-Nitrophenol (p)	Sigma Aldrich	USA
4-Nitrophenol (p)	Sigma Aldrich	USA
2,4-Dinitrophenol (p)	Sigma Aldrich	USA
2,6-Dinitrophenol (p)	Sigma Aldrich	USA

Tools and instrumentation

For the experiments on the microwave-aided phenol nitration, a common chemistry lab with a fume hood, classical laboratory equipment and tools was used. Regarding the potential hazards, the proposed methodology and chemical reactions are adequate for undergraduate university students working under supervision of a trained organic chemists

or a chemistry teacher. The crucial instruments necessary for this project are outlined in Table 2.

Table 2

Used laboratory tools and instruments

Instrument	Manufacturer	Country of origin
Laboratory scales	Kern	Germany
Kitchen microwave oven	Goddess	China
Chromatography chamber with cover glass	P-Lab	Czech Republic
Beakers of various volumes	Simax	Czech Republic
TLC plates (ALUGRAM SIL G/UV_{254})	Macherey - Nagel	Germany
Test tube	Kavalier	Czech Republic
Capillaries for TLC	Mirschmann Laborgeräte	Germany
Glass rod	Kavalier	Czech Republic
Stainless-steel test-tube stand	-	-
Hairdryer	-	-
Watch glass	-	-
Ceramic crucibles	-	-
Crucible tongs	-	-

Procedure of the proposed microwave-aided experiments

The designed microwave-aided reactions are very simple and may be performed according to the following stepwise protocol. It is presupposed that the participating students have preliminary experience with common laboratory operations like weighing solid samples, measuring of volumes, and stoichiometric calculations. In case further details of the procedure are required, do not hesitate to contact the corresponding author of this article.

Table 3

Compounds and their amounts used for the experiments

Nitrate (1)	Nitrate weight [g]	Water weight [g]
$NaNO_3$	0.09	0.17
$Ca(NO_3)_2 \cdot 4H_2O$	0.17	0.02
$Cu(NO_3)_2 \cdot 3H_2O$	0.19	0.05
$Fe(NO_3)_3 \cdot 9H_2O$	0.24	0.00
Nitrate (2)	**Nitrate weight [g]**	**Water weight [g]**
$NaNO_3$	0.13	0.22
$Ca(NO_3)_2 \cdot 4H_2O$	0.25	0.03
$Cu(NO_3)_2 \cdot 3H_2O$	0.28	0.08
$Fe(NO_3)_3 \cdot 9H_2O$	0.36	0.00
Nitrate (3)	**Nitrate weight [g]**	**Water weight [g]**
$NaNO_3$	0.18	0.34
$Ca(NO_3)_2 \cdot 4H_2O$	0.34	0.04
$Cu(NO_3)_2 \cdot 3H_2O$	0.38	0.10
$Fe(NO_3)_3 \cdot 9H_2O$	0.48	0.00
Weight of phenol [g]		
0.09		

(1) Reactant ratio: nitrate/phenol = 1.0/1.0 mol, (2) reactant ratio: nitrate/phenol = 1.5/1.0 mol, (3) reactant ratio: nitrate/phenol = 2.0/1.0 mol

1. Phenol was weighed into porcelain crucibles in the amount given in Table 3.
2. Appropriate amounts of nitrates (e.g. sodium, calcium, copper(II), iron(III) nitrates) were added to the phenol samples (Table 3).
3. 0.12 g of glacial (i.e. concentrated) acetic acid and the appropriate amount of water were added to the mixture using a plastic pipette. Additional water serves as compensation for the crystal water found in some nitrates. The water load is shown in Table 3.
4. The mixtures prepared in this way in ceramic crucibles were covered with a watch glass and placed into a kitchen microwave oven (P = 500 W), close to the centre of the rotating plate. The microwave oven was closed, and heating for two minutes was started.
5. After heating the reaction mixture, the crucibles were removed from the microwave oven with crucible tongs and allowed to cool down.
6. 0.1 cm^3 of toluene was added to the cooled reaction mixture in the crucible using a plastic pipette and the reaction mixture was thoroughly mixed with a glass rod. Samples for TLC analyses were taken from the crucibles with a glass microcapillary.

Methodology of TLC analyses

The chromatographic analyses were performed on a thin layer of silica gel (TLC) produced by Macherey-Nagel (ALUGRAM SIL G®/UV245), using toluene as an elution agent in a chromatographic chamber with a cover glass. The results of the TLC analyses are summarised in Table 4. The chromatographic analysis itself was performed according to the following general protocol:

1. On a TLC plate, start (2 cm from the bottom edge) and end (0.5 cm from the upper edge) lines were marked using a soft pencil. On the start line, markers for the prepared samples and analytical standards were made as well.
2. Samples of the analytical standards (i.e. phenols and nitrophenols) and samples of the reaction mixtures dissolved in toluene were deposited on the TLC plate by glass microcapillaries.
3. After applying the sample solutions of the prepared products to the TLC plate, the solvent was allowed to evaporate in a fume hood.
4. The TLC plate was inserted into a chromatographic chamber with a 1 cm high layer of toluene (i.e. elution agent) and the chamber was closed with a cover glass.
5. The chromatogram was left to develop with toluene.
6. The development of the chromatogram was terminated when the forehead of the mobile phase reached the end line at the top edge of the thin layer.
7. The chromatogram was removed from the chamber and dried with hot air using a hair dryer.
8. After the evaporation of the eluting agent, the chromatogram was first inserted into ammonia vapours (in a fume hood). The appeared yellow spots of nitrophenols were marked with a soft pencil.
9. Then, the chromatogram was inserted into nitrous gases prepared by decomposition of sodium nitrite with hydrochloric acid (in a fume hood). On the chromatogram, gray-green spots of unreacted phenol appeared, which were again marked with a soft pencil.
10. Finally, the chromatograms were geometrically analysed to determine the retardation factors of each studied substance. By comparison with the analytical standards, the

nitration products as well as the unreacted phenol were identified and roughly quantified.

Table 4
Values of retardation factors (R_F) of the starting substance and the reaction products (stationary phase - silica gel, elution agent - toluene)

Compound	$\boldsymbol{R_F}$
Phenol	0.20
2-nitrophenol	0.70
4-nitrophenol	0.09
2,4-dinitrophenol	0.48
2,6-dinitrophenol	0.26
2,4,6-trinitrophenol	0.05

Results and discussion

In the realm of educational experiments focused on organic chemistry, phenol nitration has an irreplaceable position. When various chemistry textbooks involving phenol nitration are analysed, the experiment is usually performed as a reaction of phenol with nitric acid or with a mixture of alkaline nitrate and sulphuric acid. Especially the second method is very popular in organic chemistry because it permits to better and more effectively influence the course of the nitration and its selectivity. Nitration based on other types of nitrates is hardly used in chemistry teaching due to reasons, which are mostly not argumentatively strong. On the other hand, the reaction of phenol with nitric acid is a straightforward educational example presenting the course of the nitration and its mechanism.

If our above-mentioned procedure is used, which utilises other nitrates than the common sodium nitrate and potassium nitrate, it is necessary to modify the relevant concepts about the nature of the reaction in a certain way. Nitration of phenol using sodium/potassium nitrate in the presence of sulphuric acid is based on the displacement of the weaker acid from its salt by the action of the stronger acid. The procedures applied in this work are more complicated both in terms of their execution in the laboratory and their theoretical interpretation. In this case, alkaline metal nitrates are supplemented by alkaline earth metal nitrates and heavy metal nitrates, sulphuric acid is replaced by acetic acid, so it is a significant innovation with respect to the existing practice of conducting an educational experiment. The main research question (RQ1) is how the nitration of phenol can take place under these conditions. The answer to this question (A1) is not very complicated, because the course of the reaction will probably be related to the type of metal that is contained in the nitrate molecule.

Before the experiments were carried out, a hypothesis about the course of the reaction was formulated, considering mainly the different structure of individual nitrates. This important part of inquiry-based learning must be properly implemented in order that the students involved may fully understand and appreciate the experiments performed. Briefly, a series of nitrates was proposed, which included both alkali metal nitrates, alkaline earth nitrates and heavy metal nitrates. The group of alkali metal nitrates was represented by sodium nitrate, which was extended by calcium nitrate as a representative of alkaline earth metal nitrates. The group of heavy metal nitrates was represented by copper(II) nitrate and iron(III) nitrate. The reaction mixture also contained concentrated acetic acid and a small amount of water. The experimental design was based on the knowledge that sodium

nitrate, calcium nitrate, copper(II) nitrate and iron(III) nitrate are salts of a strong acid and base, the strength of which decreases in the mentioned series. Especially iron(III) nitrate and copper(II) nitrate are easily hydrolysed to form acid, while sodium nitrate releases only stably solvated ions. These properties can be verified in a simple way using universal pH papers to test their aqueous solutions. In addition, the hypothesis on acid-base properties of the studied nitrates was compared with data in the literature and experimentally verified by us [25].

Therefore, the set of educational experiments was based on our previous studies and experimental verifications in the area of special didactics of organic chemistry. In the implementation of this plan, experimental procedures that have been developed in preparative chemistry in recent years were taken into account. In our case, it is a reaction in the presence of microwave radiation, carried out without a solvent. In this context, a number of experiments were designed to investigate specific factors that can influence the course of the nitration. This includes, for example, the following research topics:

1. The type of phenol used.
2. The type of nitrate used.
3. Mutual ratio of reactants.
4. The duration of microwave heating.
5. The power of microwave irradiation.
6. Location of the reaction vessel in the microwave oven.

Based on experiments, the topic (2) focused on the type of nitrate was selected for necessary didactic transformations leading to a proposal of a lesson for inquiry-based learning in organic chemistry. In principle, the task can be understood as exploring the relationships between the nitrate used and the composition of the reaction product, relying on logical reasoning and chemical abstraction.

A mechanistic analysis of the structure of substances involved in the nitration of phenol enables to propose several hypotheses about the course of the nitration reaction. As mentioned in the theoretical part, the hydroxyl group in the phenol molecule shows a positive mesomeric (+*M*) and a negative inductive (-*I*) effect. Since the positive mesomeric effect prevails over the negative induction effect, the hydroxyl group can be expected to act as an electron donor for the benzene skeleton. This results in the formation of nitrophenols, in which the nitro group is located in the *ortho* or *para* position. Therefore, the following products can be expected: 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2,6-dinitrophenol or even 2,4,6-trinitrophenol if extreme conditions are established. A crucial learning process in this task is evaluating the influence of the structure of individual nitrates on the course of nitration. In relation to the basicity of hydroxides (or hydrated oxides), the strongest nitration agent should be iron(III) nitrate, followed by copper(II) nitrate. The weakest nitration agent should be calcium nitrate, sodium nitrate should not react. It can be assumed that phenols with more nitro groups will be formed by the action of iron(III) nitrate in concentrated acetic acid, mono- and dinitrophenols by the action of copper(II) nitrate, mononitrophenols in smaller quantities by the action of calcium nitrate, and no nitrophenols should be formed by the action of sodium nitrate.

What are the results of the experiments we achieved and how do they correspond to the hypothesis? All experiments that were carried out with sodium nitrate under various conditions showed in agreement with the hypothesis that nitrophenols are not formed. In the case of calcium nitrate, nitration of the first degree takes place, among the nitration products of phenol, 2-nitrophenol and 4-nitrophenol can be identified. Copper(II) nitrate

afforded, in agreement with the hypothesis, both mononitrophenols and dinitrophenols. After phenol nitration with iron(III) nitrate and concentrated acetic acid, mononitrophenols and especially dinitrophenols were detected in the reaction product. The course of phenol nitration was controlled by thin-layer chromatography, which is a very simple analytical method broadly utilised in organic synthesis and very suitable for educational experiments in chemistry exercises. Illustration of the TLC analyses of the obtained nitration products is given in Figure 5.

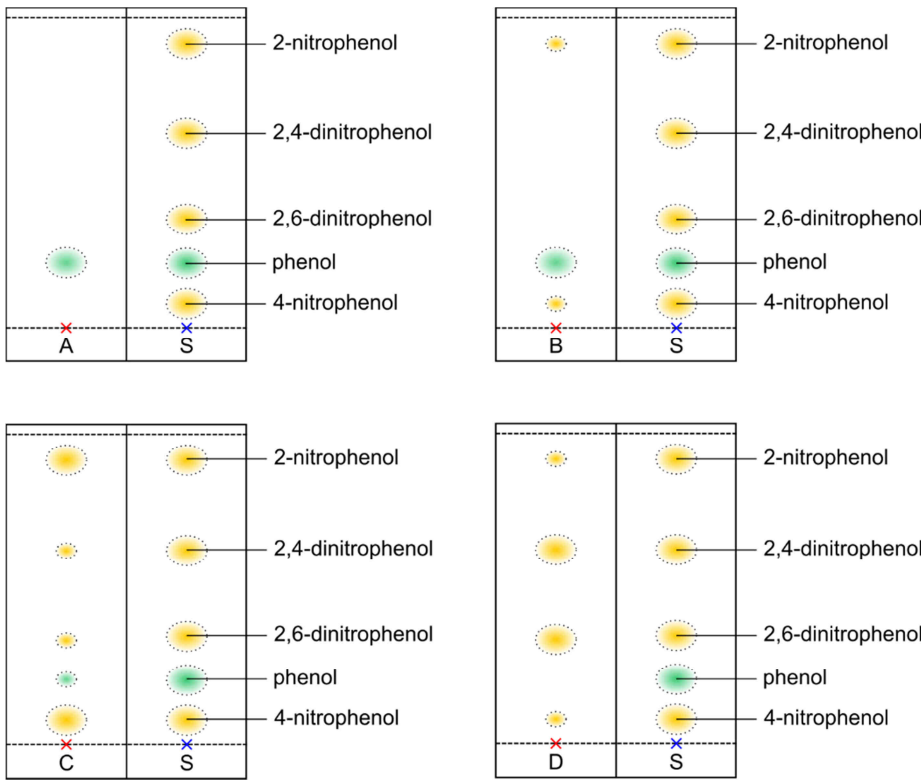

Fig. 5. Illustrative TLC analyses of the nitration products obtained with sodium nitrate (A), calcium nitrate (B), copper(II) nitrate (C), and iron(III) nitrate (D). The letter "S" denotes analysis of the analytical standards of the monitored substances. The phenol nitration by the selected metal nitrates in glacial acetic acid was supported with microwave irradiation (2 min)

Based on these findings, the following conclusions were formulated: the hypothesis on the course of nitration was confirmed, which allowed to design an interesting experimental lesson for teaching organic chemistry. The fundamental idea, which the learners should take from these experiments is: acetic acid and metal nitrates derived from strong bases do not nitrate phenol, acetic acid and nitrates derived from medium-strength bases act as nitration agents, acetic acid and nitrates derived from weak bases act as strong nitration agents. These conclusions create a starting point to elaborate instructions for experimental tasks intended for laboratory exercises in organic chemistry, analytical chemistry or for

practical training of educational experiments in university training chemistry teachers. We also plan that the instructions for the experimental task will be involved in selected practical exercises at the grammar school in the form of a workshop for students, or for a demonstration experiment carried out only by the teacher. In the future, the task will be included in practical exercises focused on educational applications of thin-layer chromatography (i.e. thin-layer chromatography and teaching chemistry - optional practical course for students of chemistry teaching). Another considered alternative is organisation of a workshop for teachers and students focused on the use of microwaves in organic chemistry (i.e. synthesis of organic compounds without solvent in the presence of microwaves - workshop for chemistry teachers and interested secondary school students).

Conclusion

The present study portrays one of the new ways towards innovative education of organic chemistry for undergraduate students. Nitration as a fundamental organic reaction was prioritised by us and transformed into an effective microwave-aided experiment, which can be easily monitored by TLC analyses. Moreover, the proposed experiments follow the principles of Green chemistry and as such they are suitable candidates for inquiry-based chemistry learning. The main ideas covered in this study may be summarised in the following points:

1. The study focused on the nitration of phenol using various metal nitrates in concentrated acetic acid and microwave irradiation.
2. In the first phase, optimisation of reaction conditions was carried out in connection with the possible use of this reaction in a specialised chemistry exercise.
3. Various factors influencing the nitration process, such as reactant quantities, microwave power, and heating time, were investigated and incorporated into the experimental design.
4. Based on the findings, a proposal of an inquiry-based learning task on the influence of nitrate structure on the course of phenol nitration was elaborated.
5. The objective of the research was to make some of the newer procedures that are used in advanced organic synthesis available for students specialising in chemistry education.

Acknowledgements

The authors would like to thank for the support of the Erasmus+ project STEMkey and the Faculty of Education Charles University Cooperatio programme.

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