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Hydrolysis of sulphonamides in aqueous solutions

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ABSTRACT

Hydrolysis is one of the most common reactions controlling abiotic degradation and is one of the main paths by which substances are degraded in the environment. Nevertheless, the available information on this process for many compounds, including sulphonamides (a group of antibiotic drugs widely used in veterinary medicine), is very limited. This is the first study investigating the hydrolytic stabilities of 12 sulphonamides, which were determined according to OECD guideline 111 (1st category reliability data on the basis of regulatory demands on data quality for the environmental risk assessment of pharmaceuticals). Hydrolysis behaviour was examined at pH values normally found in the environment. This was prefaced by a discussion of the acid–base properties of sulphonamides. All the sulphonamides tested were hydrolytically stable at pH 9.0, nine (apart from sulphadiazine, sulphachloropyridazine and sulphamethoxypyridazine) were stable in this respect at pH 7.0 and two (sulphadiazine and sulphaguanidine) at pH 4.0 (hydrolysis rate ≤ 10%; $t_{0.5\,(25\,^{\circ}C)}$ > 1 year). The degradation products were identified, indicating two independent mechanisms of this process. Our results show that under typical environmental conditions (pH and temperature) sulphonamides are hydrolytically stable with a long half-life; they thus contribute to the on-going assessment of their environmental fate.

1. Introduction

The majority of the earth's surface is covered by water in the form of oceans, seas, lakes or rivers. Hence, chemical pollutants (e.g. pharmaceuticals) entering the environment are usually degraded via hydrolysis [1]. Because water is present in great excess compared to the concentrations of the chemicals, this type of reaction is usually described as a pseudo-first order reaction at fixed pH and temperature, and may be influenced by acidic or basic species H_3O^+ and OH^-. It has been established, that many of the most frequently applied penicillins are difficult to detect in the environment because they are hydrolysed, especially in alkaline sewage [2,3].

Determination of the hydrolysis behaviour of pharmaceuticals is especially important in the case of poorly or non-biodegradable substances. This is because, for example, the presence of antibiotics in the environment may elicit the development of antibiotic-resistant genes in microorganisms, which can be transferred to human beings and animals through food chains and drinking water, resulting in the failure of antibiotic treatment of infections [4,5]. On the other hand, when pharmaceuticals degrade in the environment, they may form persistent and toxic transformation products, which should be accounted for in the environmental risk assessment (ERA) of the parent compounds [6]. Even though hydrolysis is known to be one of the most common chemical reactions controlling stability and is, therefore, one of the principal chemical transformation pathways of these substances in the environment, literature data on the hydrolytic stabilities of pharmaceuticals are very limited [7].

In order to arrive at reliable ERAs, suitable data on the environmental exposures and ecotoxic potencies of compounds are needed [8]. Regulatory demands on data quality for the ERA of veterinary pharmaceuticals are given in the guideline on environmental impact assessment for veterinary medicinal products [9]. According to the European Medicines Agency (EMEA) and the Food and Drug Administration (FDA), the laboratory test method for assessing abiotic hydrolytic transformations of chemicals in aquatic systems at pH values normally found in the environment (pH 4–9) should be based on OECD Guideline 111 [10,11].

Sulphonamides (SAs) are antibiotics that have been widely used in veterinary medicine for almost fifty years [12,13]. They are incompletely metabolised and are excreted partly as unchanged parent compounds and partly as metabolites [14]. They enter the ecosystem from wastewater discharge, manure disposal, aquaculture and animal grazing [15], and may be later transported to various environmental compartments such as surface water,

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ground water, soil or sludge. Sukul and Spiteller [16] noted that during the storage of manure, the excreted acetyl conjugates could be cleaved back to the parent compounds. SAs are only partially removed by conventional WWTPs [17,18], and are found in surface water (in the ng L^{-1} to μg L^{-1} range) and ground water (ng L^{-1}) [e.g. 19,20]. Such distribution may have significant and long-term effects on the rate and stability of ecosystem functioning [12,21–24].

The literature data concerning the hydrolytic stabilities of sulphonamides are contradictory. On the one hand, they indicate that these antibiotics are resistant to hydrolysis [25–28], on the other, they claim that they are degradable, especially in acidic solution [29–32]. Moreover, the hydrolysis rates were calculated using procedures other than OECD 111.

In this work, the hydrolysis behaviour of 12 sulphonamides was investigated according to OECD 111. The formation of selected degradation products – aniline, sulphanilic acid and sulphanilamide – was monitored and the mechanism of sulphonamide hydrolysis discussed. Reliable hydrolytic stability data for all the tested SAs were obtained.

2. Materials and methods

2.1. Chemicals

All sulphonamides (Table 1), apart from sulphadimidine and sulphapyridine (Serva, Heidelberg, Germany), were purchased from Sigma–Aldrich (Steinheim, Germany). The compounds used for preparing buffer solutions (all analytical grade) – monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), potassium chloride (KCl) and boric acid (H_3BO_3) – were obtained from POCH (Gliwice, Poland), and citric acid ($C_6H_3O_7 \times H_2O$) and sodium hydroxide (NaOH) were from Stanlab (Lublin, Poland). Deionised water was produced by the HYDROLAB System (Gdansk, Poland). The acetonitrile (ACN) (HPLC grade) used for the mobile phases and for preparing the comparative standard solutions was obtained from POCH S.A. (Gliwice, Poland). Trifluoroacetic acid 99% (TFA) (for mobile phase acidification) was purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. The hydrolytic stabilities of 12 sulphonamides determined according to OECD 111

The hydrolytic stabilities of the SAs were determined according to the method set out in OECD 111 [10]; the scheme is shown in Fig. 1. The studies were conducted in capped glass vials, under dark and sterile conditions. Before and after degradation, the samples were analysed using a validated HPLC-UV method [33] in order to quantify the test substances and hydrolysis products. All tests were done in two replicates. Chromatographic separations were performed using a high performance liquid chromatograph (Perkin Elmer Series 200) consisting of a chromatographic interface (Link 600), binary pump, UV/vis detector, vacuum degasser and Rheodyne injection valve. SA samples were separated on a Gemini C18-110A column (150 mm × 4.6 mm, 5 μm, Phenomenex Inc. Torrance, CA) (room temperature, wavelength 270 nm, injection volume 50 μL, flow rate 0.7 mL min^{-1}). Mobile phase A was H_2O with the addition of TFA at pH 3.5, and mobile phase B was 100% acetonitrile; both were selected in the gradient programme. Elution began with 90% of mobile phase A, which was reduced to 40% within 20 min. Chromatographic separation of SAs and degradation products was achieved within less than 20 min.

2.2.1. Test conditions

Experiments were performed at pH 4.0, 7.0 or 9.0.

Buffer solution pH 4.0 was prepared from aqueous solutions 0.2 M K_2HPO_4 and 0.1 M citric acid mixed in the ratio 38.55/61.45 (v/v). The pH 7.0 buffer consisted of 0.1 M NaOH, 0.1 M KH_2PO_4 and water in the ratios 29.63/50.00/20.37 (v/v/v), and the pH 9.0 buffer of 0.1 M NaOH, 0.1 M H_3BO_3 in 0.1 M KCl and H_2O in the ratios 21.30/50.00/28.70 (v/v/v). The pH of each buffer solution was checked with a CP-411 laboratory pH-metre (Elmetron-Zabrze, Poland) to an accuracy of at least 0.1 at the required temperature. Next, the buffer solutions were passed through 0.2 μm fibreglass (Chromafil® 148 PET 15/25, Marchery-Nagel, Düren, Germany), bubbled by nitrogen for 5 min (to avoid oxygen) and thermostated at the required temperature before the experiment.

2.2.2. Performance of the test

2.2.2.1. Preliminary test (Tier 1). On the day of the experiment, a stock solution of the sulphonamide to be analysed (100 mg L^{-1}) was added to the appropriate buffer solution (bubbled again by nitrogen for 5 min) to a concentration of SA 1 mg L^{-1}. The solution obtained was divided into two portions: the first portion was subjected to HPLC-UV analysis, whereas the second one was transferred to a 10 mL glass vial (typical of the headspace technique) and stored in a capped vial under dark and sterile conditions at the required temperature and for the requisite length of time (Fig. 1) (Incubator ICT 5.4, Falc, Treviglio, Italy). Additionally, on the day of the experiment, the control solution (1 mg L^{-1}) of SA in a mixture of H_2O:ACN (90:10, v/v) was prepared. It was stored at 4 °C, then analysed by HPLC-UV on the first day of the experiment; subsequently, the degraded sample was likewise subjected to HPLC-UV analysis on selected days (Fig. 1). This procedure was applied to each sulphonamide.

Determination of the hydrolysis rate of the SAs was based on Eqs. (1)–(3):

$$S^0 = \frac{P^0}{M^0} \times 100 \quad (1)$$

$$S^t = \frac{P^t}{M^t} \times 100 \quad (2)$$

$$S^0 = S^0 - S^t \quad (3)$$

where S is the SA hydrolysis rate [%], S^0 is the hydrolysis rate of SA before hydrolysis [%], S^t is the hydrolysis rate of SA determined for a time t degraded sample [%], P^0 is the chromatographic peak area of SA determined for a non-degraded sample on the day of the experiment, P^t is the chromatographic peak area of SA after sample degradation at fixed pH, temperature and time, M^0 is the chromatographic peak area of SA determined for the control solution on the first day of the experiment, M^t is the chromatographic peak area of SA determined for the control solution kept for time t at 4 °C.

2.2.2.2. Hydrolysis of unstable substances (Tier 2). The higher tier test was performed at the pH values at which the test substance was found unstable, as defined by the preliminary test above (Fig. 1). The buffered solutions of the test substance were thermostated at selected (20 °C, 40 °C and 70 °C) temperatures. To test the hydrolysis rate as a function of pH and temperature, each reaction was allowed to proceed for 30 days, and individual replicate test samples (in separate reaction vessels) were analysed by HPLC-UV ($n = 3$) at each of six sampling times. Next, for each hydrolytically unstable SA, the following function (4) was applied in order to test kinetic behaviour:

$$k_{obs} = \frac{1}{t} \times \ln \frac{C_t}{C_0} \quad (4)$$

where k_{obs} is the pseudo first-order hydrolysis rate constant at fixed temperature ($time^{-1}$), C_0 and C_t are the respective concentrations of the SA at time zero and t, ln is the Naperian logarithm. For each sulphonamide, the logarithms of the concentrations $\ln C_t/C_0$ were plotted against time (t) and the slope of the resulting straight line

Table 1
Structures and selected properties of the sulphonamides investigated (according to [16,34–43]).

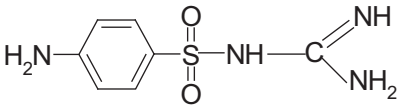
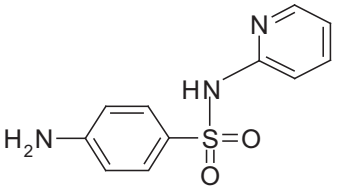
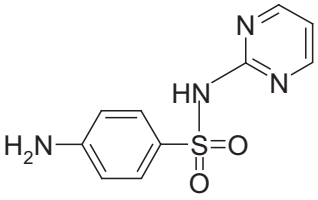
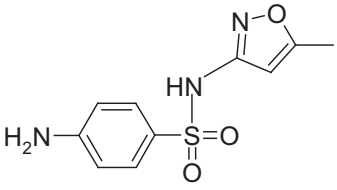
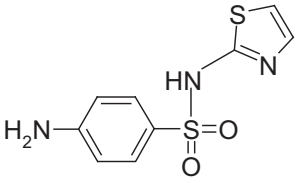
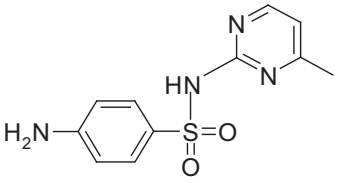
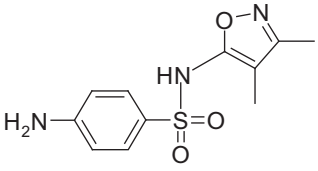
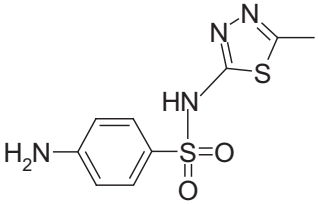
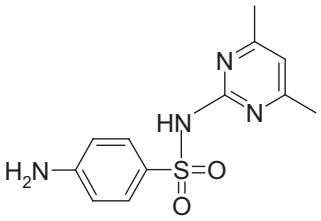
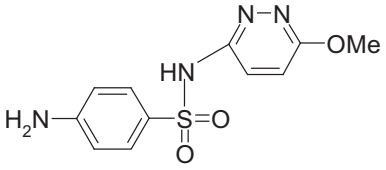
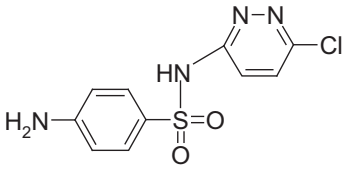
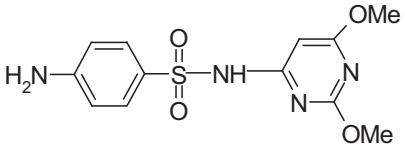
Substance [CAS]	Chemical structure	Selected physico-chemical properties
Sulphaguanidine [57-67-0]		M = 214.2 g mol^{-1} pK_{a1} = 0.5 pK_{a2} = 2.8 pK_{a3} = 12.1 log P = −1.22 S_{H_2O} = 2.19 g L^{-1}
Sulphapyridine [144-83-2]		M = 249.2 g mol^{-1} pK_{a2} = 2.4 pK_{a3} = 8.2 log P = 0.35 S_{H_2O} = 0.50 g L^{-1}
Sulphadiazine [68-35-9]		M = 250.3 g mol^{-1} pK_{a2} = 1.8 pK_{a3} = 6.5 log P = −0.09 S_{H_2O} = 0.08 g L^{-1}
Sulphamethoxazole [723-46-6]		M = 253.3 g mol^{-1} pK_{a2} = 1.8 pK_{a3} = 5.7 log P = 0.89 S_{H_2O} = 0.61 g L^{-1}
Sulphathiazole [72-14-0]		M = 255.3 g mol^{-1} pK_{a2} = 2.1 pK_{a3} = 7.1 log P = 0.05 S_{H_2O} = 0.95 g L^{-1}
Sulphamerazine [127-79-7]		M = 264.3 g mol^{-1} pK_{a2} = 1.8 pK_{a3} = 6.8 log P = 0.14 S_{H_2O} = 0.37 g L^{-1}
Sulphisoxazole [127-69-5]		M = 267.3 g mol^{-1} pK_{a2} = 1.8 pK_{a3} = 5.0 log P = 1.01 S_{H_2O} = 0.33 g L^{-1}
Sulphamethiazole [144-82-1]		M = 270.3 g mol^{-1} pK_{a2} = 1.8 pK_{a3} = 5.45 log P = 0.54 S_{H_2O} = 1.05 g L^{-1}

Table 1 (*Continued*)

Substance [CAS]	Chemical structure	Selected physico-chemical properties
Sulphadimidine(sulphamethazine) [57-68-1]		M = 278.3 g mol^{-1} pK_{a2} = 2.5 pK_{a3} = 7.4 log P = 0.89 S_{H_2O} = 1.49 g L^{-1}
Sulphamethoxypyridazine [80-35-3]		M = 280.3 g mol^{-1} pK_{a2} = 2.2 pK_{a3} = 7.2 log P = 0.32 S_{H_2O} = 0.15 g L^{-1}
Sulphachloropyridazine [80-32-0]		M = 284.7 g mol^{-1} pK_{a2} = 2.2 pK_{a3} = 5.5 log P = 0.31 S_{H_2O} = 7.00 g L^{-1}
Sulphadimethoxine [122-11-2]		M = 310.3 g mol^{-1} pK_{a2} = 2.5 pK_{a3} = 5.9 log P = 1.63 S_{H_2O} = 0.34 g L^{-1}

was calculated. A high value of the correlation coefficient (R^2) (~1) indicated first-order or pseudo-first order behaviour of the reaction tested, and the slope of the resulting linear regression yielded the rate constant. Then, the half-life of the test ($t_{0.5}$) substances was calculated using the following equation (5):

$$t_{0.5} = \frac{\ln 2}{k_{obs}} \quad (5)$$

2.2.2.3. Analysis of hydrolysis products (Tier 3). The formation of selected SA hydrolysis products – aniline, sulphanilic acid and sulphanilamide – was investigated by HPLC-UV under the chromatographic conditions previously applied to the parent compounds [33]. These products were identified on the basis of their retention parameters, established by analyses of the stock solutions (1 mg L^{-1}) of these compounds in a H_2O:ACN mixture (90:10, v/v) performed under the same chromatographic conditions.

3. Results and discussion

The chemical structure, CAS number and selected physico-chemical properties of the compounds analysed are given in Table 1 [16,34–43]. Since the behaviour of SAs in aqueous solution at different pH values depends on their acid–base properties, they should be discussed first.

3.1. Acid–base properties of sulphonamides

All the sulphonamides, apart from sulphaguanidine, which will be discussed separately, are compounds with two basic and one acidic functional group. The basic functional groups are the amine group of aniline (all the SAs) and the respective heterocyclic base, specific to each SA. The acidic functional group in the SAs is the sulphonamide group, which is known to lose its proton relatively easily ($pK_a \approx 5$–8). With such an SA structure, these compounds may be described by the pK_{a1}, pK_{a2} and pK_{a3} values corresponding to the double protonated (I), once protonated (II) and neutral forms of SA (III, Fig. 2). The double protonated form of SA (I) is a strong acid with a very low pK_{a1} (<2), so this exists only under very acidic conditions. For these reasons, the pK_{a1} values of SAs are not usually mentioned, although some of them were established [42]. Table 1 lists the pK_{a2} and pK_{a3} values for all the SAs tested, except for sulphaguanidine, which is additionally characterised by a pK_{a1} value [16,34–43]. The pK_{a2} value is usually ascribed to the aniline amino group (Fig. 2) [43], probably because this is regarded as a stronger base (aniline pK_b 9.4) than the heterocyclic bases (e.g. pyrimidine pK_b 13) [41]. However, the site of the first protonation in SAs does not always seem to be as obvious, because there are different heterocyclic bases (e.g. pyridine pK_b 8.74). Also to be taken into account is the fact that the sulphonamide group in the *para* position diminishes the basicity of aniline, whereas in the *ortho* position, this group increases the basicity of the heterocyclic amines. Undoubtedly, the pK_{a2} values presented in Table 1, ranging from 1.8 to 2.8, indicate that the once-protonated base in the SAs is rather weak, with pK_b about 11–12. Similarly, the pK_{a3} values presented in Table 1, ranging from 5.0 to 8.2, confirm the relatively strong acidic properties of the sulphonamide group in the SAs.

As mentioned, sulphaguanidine is unusual compared to the other SAs that we tested because it has no acidic functional group (pK_{a3} 12.1). If sulphaguanidine were a typical SA (V, Fig. 3), it would possess a sufficiently acidic sulphonamide proton (pK_{a3}, Table 1) and a sufficiently basic guanidine group ($pK_b \sim 1$) to exist as a

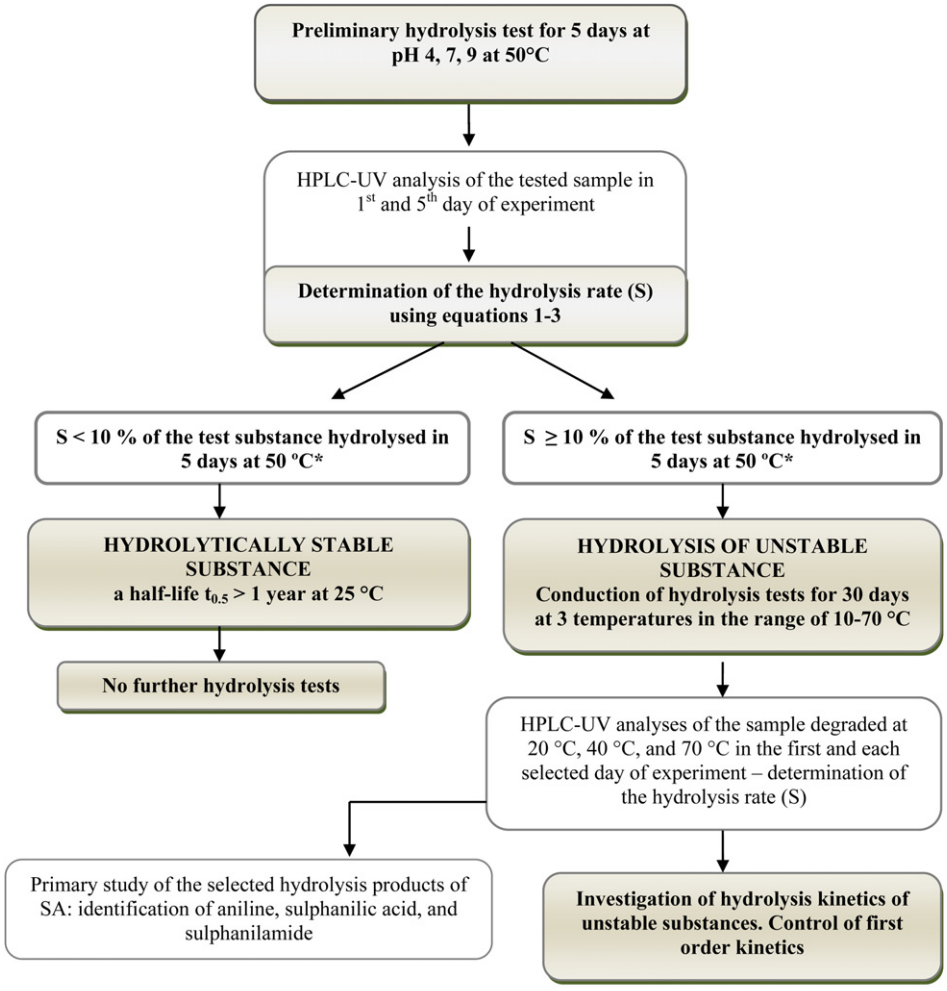


Fig. 1. General scheme of the procedure for determining the hydrolytic stabilities of 12 sulphonamides based on the OECD 111 procedure.

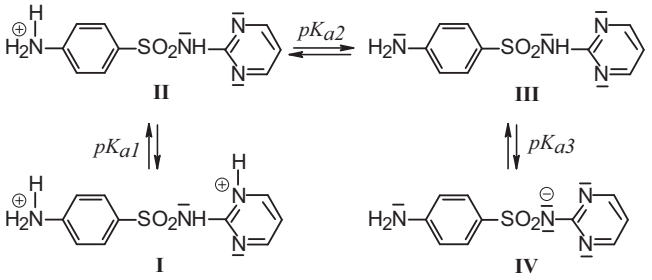


Fig. 2. Forms of sulphapyridine observed at different pH values.

zwitterion (VI). Such a zwitterion should be exceptionally stabilised by resonance, which results in structure VII of sulphaguanidine, as demonstrated by IR, ^{1}H, ^{13}C, and ^{15}N NMR [44]. This structure explains the typical acidic properties of sulphaguanidine. Its aniline amino group is comparably basic to the other SAs (pK_{a2} 2.8) but its guanidine group is only very weakly basic (pK_{a1} 0.5). In the neutral form (X, Fig. 4) sulphaguanidine has no sulphonamide proton, and so has very weak acidic properties (pK_{a3} 12.1).

3.2. Preliminary test of the hydrolytic stabilities of 12 sulphonamides

The preliminary tests were performed for 5 days at 50 ± 0.1 °C and pH 4.0, 7.0 and 9.0 (Section 2.2.2.1, Fig. 1). The results for all SAs are presented in Fig. 5. According to OECD 111 [10], if hydrolysis is less than 10% after 5 days, the test substance is considered hydrolytically stable and usually no further testing is required ($t_{0.5\,(25\,^{\circ}C)}$ > 1 year). The black line in this figure represents the borderline value of the hydrolysis rate (S) – 10%.

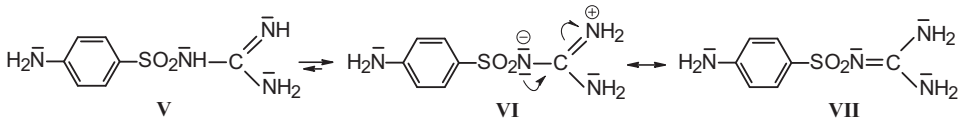


Fig. 3. Possible structures of sulphaguanidine (VII) is the proper one.

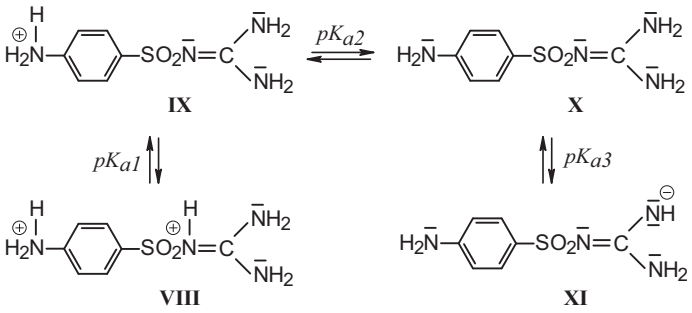


Fig. 4. Forms of sulphaguanidine observed at different pH values.

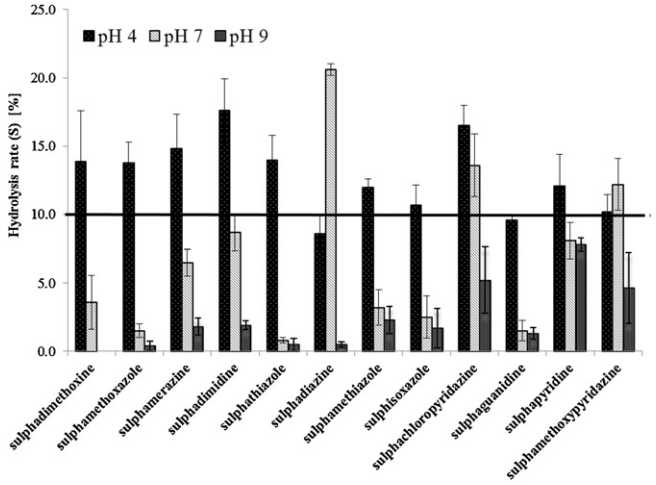


Fig. 5. Results of preliminary tests for determining the hydrolytic stabilities of 12 sulphonamides.

In general the hydrolysis rate for all SAs at 50 °C after 5 days was the highest at pH 4 and the lowest at pH 9.0. As the hydrolysis rates of all SAs at pH 9.0 were less than 10% (Fig. 5), all are assumed to be hydrolytically stable at this pH at 25 °C for least 1 year. These results were in agreement with the observations of other scientists [28,29,45,46]. This can be explained by the presence of anionic forms of SAs in alkaline solution [29] that are less sensitive to hydrolysis than the neutral and cationic forms of these compounds (pK_{a2} and pK_{a3} of SAs are listed in Table 1). Similar results were observed for sulphonylureas (compounds containing sulphonamide groups), which were hydrolysed almost 1000 times faster when neutral water molecules attacked the neutral forms of these compounds than when hydroxyl groups (OH^-) attacked their anionic forms [45].

The decrease in pH of aqueous solutions from 9.0 to 7.0 caused a decrease in the anionic forms, and an increase in the neutral and cationic forms of SAs. For these reasons, nine of the twelve compounds were hydrolytically stable at pH 7.0, whereas only two of them (sulphadiazine and sulphaguanidine) were hydrolytically stable in an acidic environment (pH 4.0). At pH 7.0 sulphadiazine (20.6%), sulphachloropyridazine (13.6%) and sulphamethoxypyridazine (12.2%) were hydrolytically unstable, whereas at pH 4.0, sulphadimidine, sulphamerazine and sulphachloropyridazine among the 10 unstable SAs exhibited the highest level of degradation (S from 14.6% to 17.6%, Fig. 5). These results indicate that SAs with six-membered heterocyclic rings are more easily hydrolysed than those with five-membered heterocyclic rings. Of all the SAs tested, sulphaguanidine was found to be the hydrolytically most stable compound. These facts will be discussed below, together with the postulated mechanisms. Hydrolysis rates at pH 4 are higher because under strongly acidic conditions sulphonamides are present mainly in the cationic form, which is more susceptible to hydrolysis than the neutral and anionic forms of these compounds [29,31].

3.3. Hydrolytic behaviour of SAs defined as unstable by the preliminary test

The sulphonamides defined as unstable by the preliminary test were subjected to further investigations according to OECD guideline 111 [10]. The tests were conducted for 30 days at 20 °C, 40 °C and 70 °C at the pH values at which the test compounds were found to be unstable (Section 2.2.2.2). The kinetics of SA hydrolysis at fixed pH and temperature were determined. Fig. 6 shows the hydrolysis rates of sulphonamides as a function of temperature (20 °C, 40 °C and 70 °C) at pH 4.0.

The results confirmed that SA hydrolysis was closely dependent on temperature. The hydrolysis rate of each SA increased with rising temperature, but after the recommended 30-day degradation period [10], these values were only slightly higher than those found at 50 °C. At pH 4.0 and at 70 °C hydrolytic degradation was the highest for sulphachloropyridazine (41%), sulphadimidine (36%), sulphisoxazole (28%) and sulphamerazine (22%), and the least for sulphamethoxazole and sulphamethiazole (< 12%). At pH 4.0 and 20 °C the hydrolytic degradation of all the SAs was 12% less after 30 days (Fig. 6).

The hydrolytic degradation of sulphadiazine, sulphamethoxypyridazine and sulphachloropyridazine was also low when hydrolysis was carried out at pH 7.0 and 20 °C, 40 °C or 70 °C (data not shown). For example, hydrolysis rates of sulphadiazine after 30 days' degradation at all three temperatures were less than 20%.

As already mentioned, the kinetics of hydrolysis is generally pseudo-first order at fixed pH and temperature. This means that the rate of disappearance of the test substance is directly proportional to the concentration of the test substance and is not a function of the concentration of any other substance present in the reaction mixture. In the next step of this study, in order to test the kinetics of SA hydrolysis, the logarithms of the concentrations of SAs $\ln C_t/C_0$ were plotted against time (t) and the slopes of the resulting straight lines were analysed (for example, Fig. 7). As already mentioned, when the correlation coefficient (R^2) is around unity, the kinetic behaviour is first-order or pseudo-first order, but when it is significantly different from unity, the reaction does not conform to such kinetic behaviour. The results confirmed that for many SAs these functions were not rectilinear (data not show). Fig. 7 shows all the correlations with R^2 close to unity.

The rate constants of the hydrolysis reaction (k_{obs}) and the half-lives ($t_{0.5}$) of SAs were determined (according to equations 4 and 5) only for compounds hydrolysing by a first-order or a pseudo-first order reaction ($R^2 > 0.92$) (Fig. 7). Therefore, k_{obs} at 20 °C and pH 4.0 for sulphadimethoxine and sulphadimidine were 0.0027 d^{-1} and 0.0038 d^{-1}, respectively, whereas k_{obs} for sulphamerazine at 40 °C and pH 4.0 was 0.0085 d^{-1}. The half-lives of these compounds ($t_{0.5}$) in the environment at the above-mentioned temperature and pH were 257 days for sulphadimethoxine, 182 days for sulphamerazine and 81 days for sulphadimidine.

The lack of a linear correlation between $\ln C_t/C_0$ and t for 7 sulphonamides could be due to the poor degradation of sulphonamides under the conditions applied (Section 2.2.2.2). Although 10 of the 12 sulphonamides were found to be unstable in the preliminary tests, the hydrolysis rates of the majority of the sulphonamides tested rose only during the first 15 days of the experiment and were similar to or only slightly higher than the

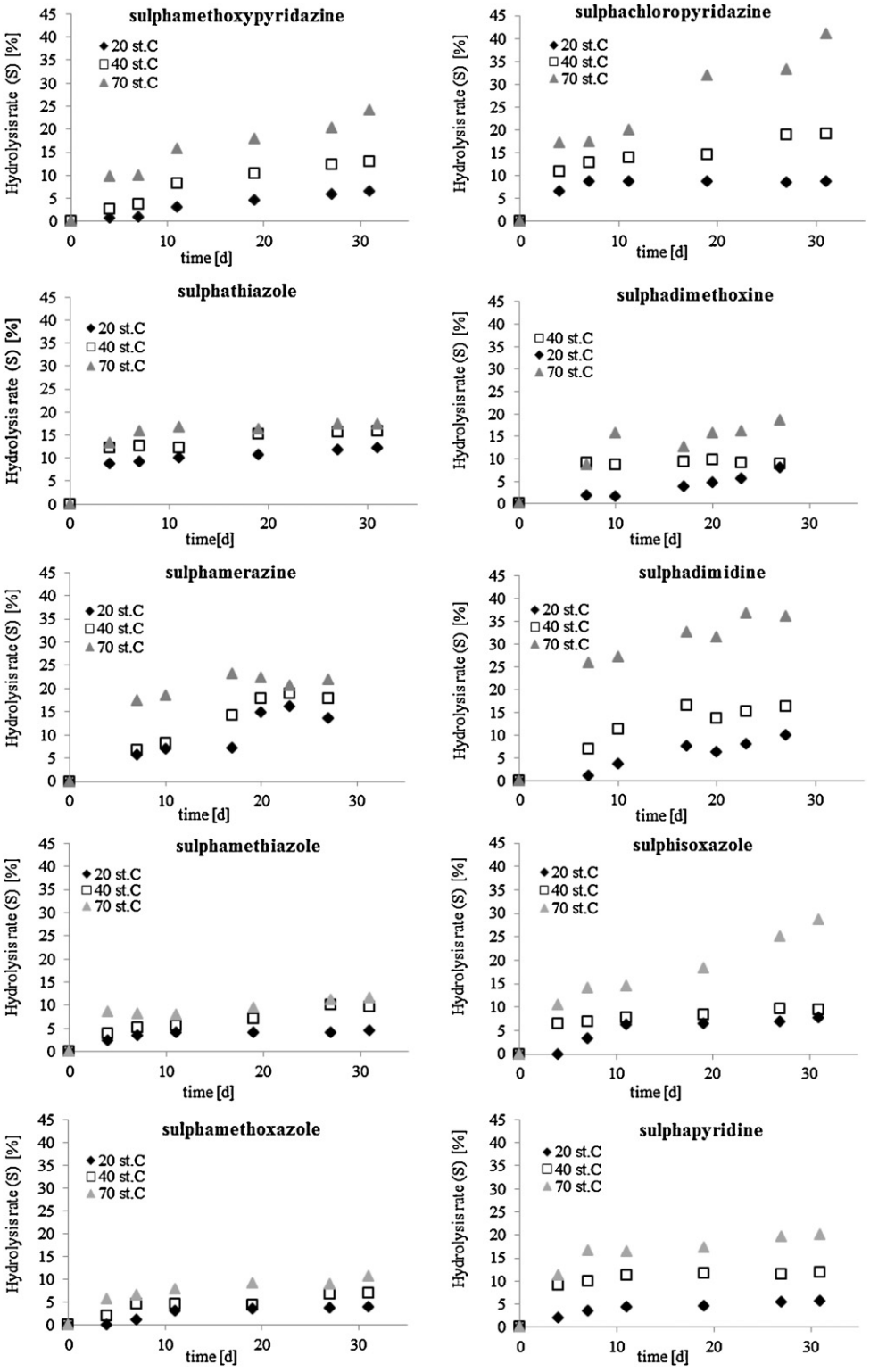


Fig. 6. The hydrolysis rates of sulphonamides as a function of temperature (20 °C, 40 °C and 70 °C) at pH 4.0.

values on the 30th day of tests (Fig. 6). This means that hydrolysis of sulphonamides takes place only very slowly even at 70 °C. The lack of a linear correlation between $\ln C_t/C_0$ and t for 7 sulphonamides could also be due to the possibility that two different reactions, both with pseudo-first order kinetics, are resposible for SA hydrolysis. The products of sulphachloropyridazine hydrolysis – sulphanilic acid and sulphanilamide (decribed below) – confirm the probability that in the case of this SA, hydrolysis takes place via two independent pathways.

3.4. Identification of SA hydrolysis products

According to literature data, the main products of sulphonamide hydrolysis are sulphanilic acid, sulphanilamide and aniline

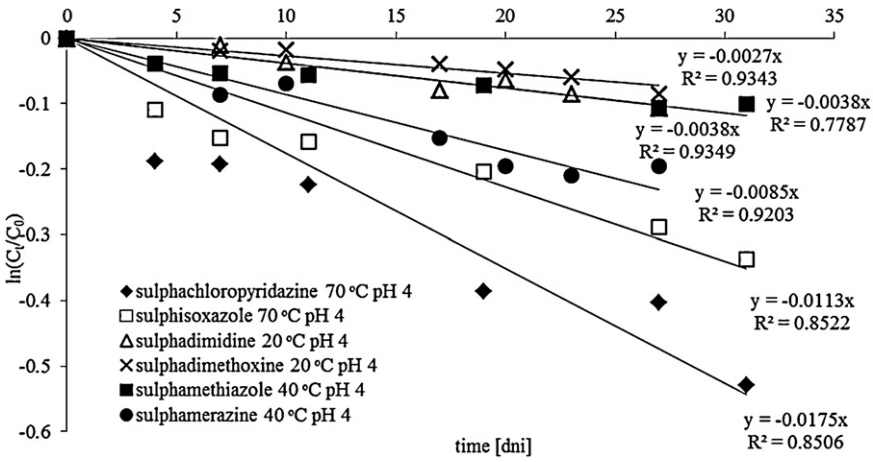


Fig. 7. A graphical presentation of the log-transformed data of the tested SA concentrations ($\ln C_t/C_0$) against time (t).

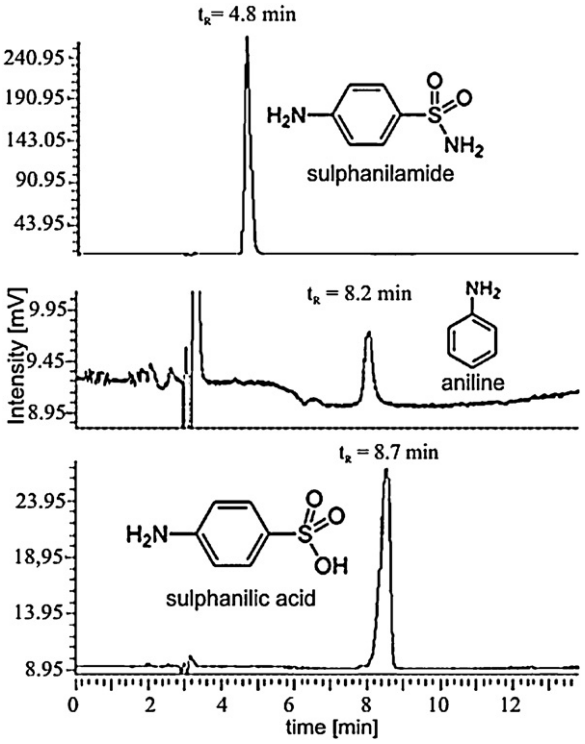


Fig. 8. Chromatograms obtained from HPLC analyses of stock solutions (1 mg L^{-1}) of sulphanilamide, aniline and sulphanilic acid in an H_2O:ACN mixture (90:10, v/v). Chromatographic conditions: Gemini C18-110A (150 mm × 4.6 mm, 5 μm), mobile phase flow rate 0.7 mL min^{-1}. Mobile phase A: H_2O with added TFA at pH 3.5; mobile phase B: 100% acetonitrile. Elution began with 90% of mobile phase A, which was reduced to 40% within 20 min.

[30,31,46]. For this reason, their formation in the degraded samples was monitored. These products were identified on the basis of the retention times of their chromatographic peaks, established by HPLC analyses of stock solutions of sulphanilamide, aniline and sulphanilic acid under the chromatographic conditions previously applied to the parent compounds (Section 2.2.2.3) (Fig. 8). The chromatograms obtained from HPLC analyses of the sulphonamides before and after 30 days' hydrolysis at 70 °C and pH 4 are presented in Fig. 9.

Sulphanilic acid, sulphanilamide or aniline were identified as the degradation products of sulphisoxazole, sulphadimethoxine, sulphamethoxypyridazine and sulphachloropyridazine only when hydrolysis was performed at pH 4.0 and 70 °C (Fig. 9). Hydrolysis of SAs conducted at lower temperatures (20 °C and 40 °C) did not yield these products, the main reason probably being the insufficient sensitivity of HPLC-UV to such very slightly degraded parent compounds. On the other hand, there were additional chromatographic peaks on the chromatograms of the 30-day hydrolysed samples. In the future, degraded samples should be analysed by LC–MS/MS in order to identify all SA hydrolysis products.

3.5. Mechanisms of sulphonamide hydrolysis

In the context of the products obtained, two possible mechanisms of SA hydrolysis may be postulated. Although they are discussed with reference to sulphachloropyridazine (Figs. 10 and 11), they are also applicable to the other SAs. The first mechanism resembles a nucleophilic acyl substitution but which takes place in the sulphonyl group. Sulphanilic acid and the corresponding heterocyclic base with an amine group are the products of such a hydrolysis. This kind of substitution is rather unlikely in view of the basic nature of the leaving groups, such as 6-chloro-3-pyridazinamine (Fig. 10). This reaction is therefore unlikely under neutral conditions, even if it takes place at higher temperatures. Altering the hydrolysis conditions from neutral to acidic should facilitate this reaction for two reasons: firstly, the protonated substrate attracts the nucleophilic water molecule more easily; secondly, under acidic conditions the leaving group is a neutral molecule (under basic conditions the eliminated amino-heterocycles are negatively charged), which makes for their easier substitution. However, the equilibrium of the acid-catalysed hydrolysis of SAs is still shifted towards the substrates, since the reverse elimination of the intermediately substituted water molecule is much easier than the elimination of the corresponding base. Under basic conditions, the anionic form IV of SAs (Fig. 2) is dominant, which causes the nucleophilic hydroxide ion to be repelled rather than attracted by the hydrolysed molecule.

Aniline, also an SA hydrolysis product, is probably released from the analogous nucleophilic sulphonyl substitution shown in Fig. 10. Undoubtedly, this is the least suitable leaving group in the intermediate, so such hydrolysis appears to be impeded. However, our own results and those of others [30,46] confirm that it is possible to eliminate aniline from SA at high temperature under acidic conditions.

The second of the proposed mechanisms of SA hydrolysis is aromatic nucleophilic substitution, which takes place in the heterocyclic aromatic ring (Fig. 11). Generally, such a substitution requires a suitable leaving group and substituents that withdraw electrons. It seems that the sulphamide group, being a relatively weak base,

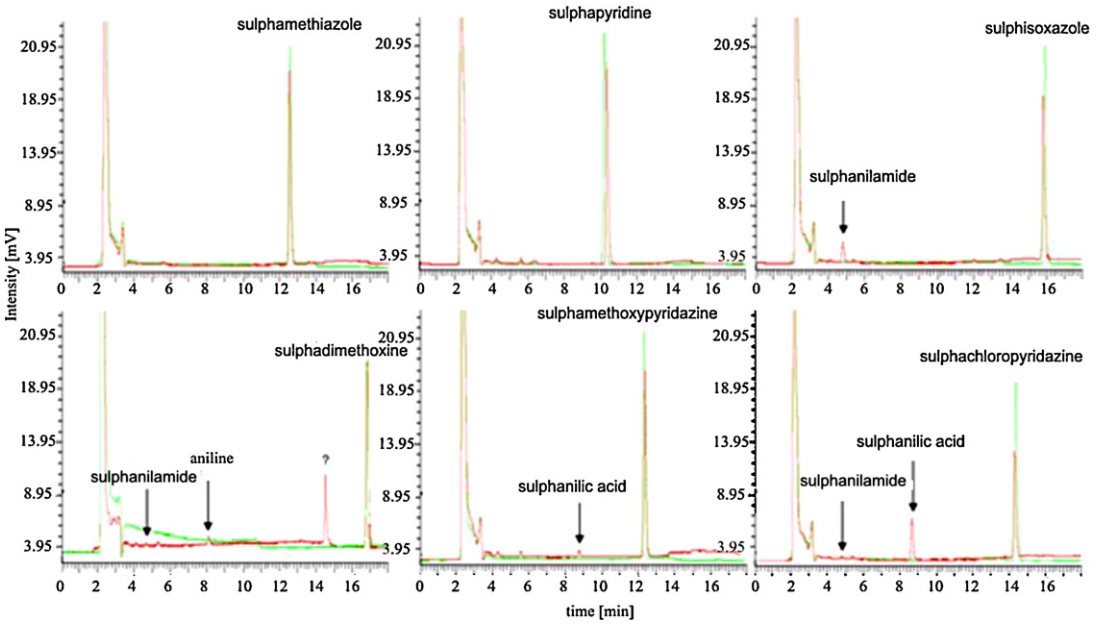


Fig. 9. Chromatograms obtained from HPLC analyses of sulphonamides before (green) and after (red) 30 days' hydrolysis at 70 °C and pH 4. Chromatographic conditions identical as presented in Fig. 8 legend. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

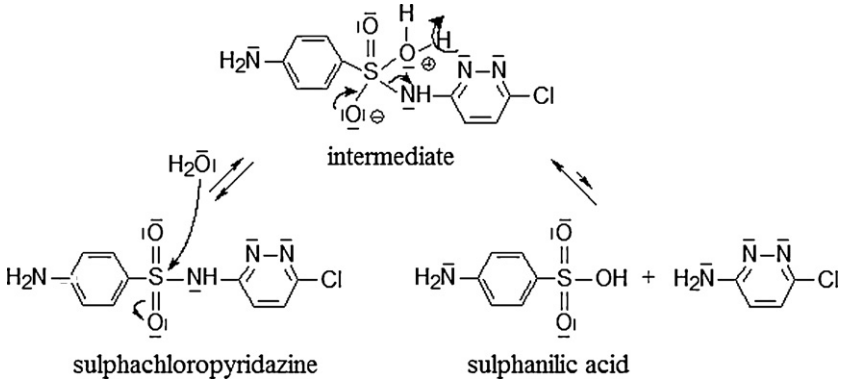


Fig. 10. Nucleophilic sulphonyl substitution under neutral conditions; the first of the postulated mechanisms of SA hydrolysis.

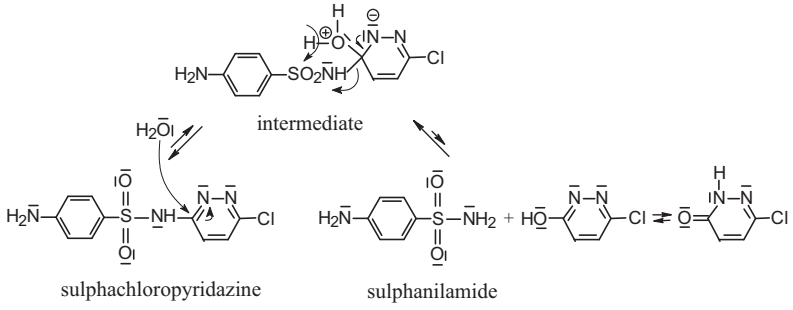


Fig. 11. Nucleophilic aromatic substitution under neutral conditions; the second of the postulated mechanisms of SA hydrolysis.

is suitable for elimination from the intermediate shown in Fig. 11. On the other hand, some of the heteroatoms in aromatic rings, particularly six-membered ones, may act as electron-withdrawing groups. As in the case of the first mechanism, acidic conditions should facilitate this substitution, whereas basic conditions preclude this substitution because of the anionic form of SA.

It is well known that six-membered rings are more reactive than five-membered ones towards aromatic nucleophilic substitution [41]. The results presented here confirm this statement, because SAs with azole rings are much more resistant to hydrolysis than those with pyrimidine, pyridazine or pyridine rings. Of course, SAs with azole rings may hydrolyse according to the nucleophilic sulphonyl substitution, the first postulated mechanism of SA hydrolysis. But comparison of the respective leaving groups suggests that compounds with an amine group bound to a six-membered ring, especially in the *ortho* position in relation to the heterocyclic nitrogen atom, should be better stabilised by resonance than compounds with an amine group bound to an azole

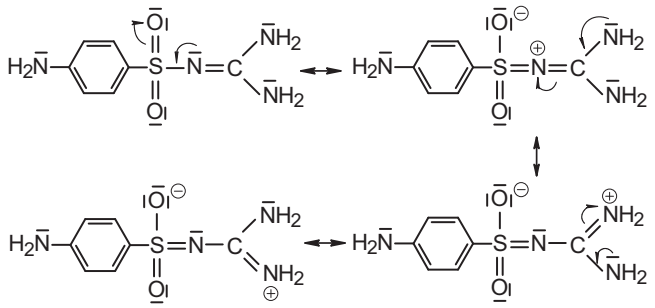


Fig. 12. The resonance stabilisation of sulphaguanidine.

ring. Such stabilisation makes six-membered heterocycles with an amine group better leaving groups in nucleophilic sulphonyl substitution. Again, these theoretical considerations are in agreement with the results presented here.

Sulphaguanidine is an exceptional compound among the SAs we tested. It does not contain an aromatic heterocyclic ring and exists mainly in neutral form at all the pH values we tested. It is also the most resistant to hydrolysis, even at high temperature. Such a lack of reactivity is probably due to the unusual stability of the neutral form of sulphaguanidine. Typical for the all SAs delocalisation of the nitrogen lone pair of electrons on the sulphonyl oxygen takes place much willingly in the case of sulphaguanidine because the positive charge is readily delocalised onto the both amine groups (Fig. 12).

3.6. Probability of sulphonamide hydrolysis in the environment

On the basis of these studies and the knowledge that the pH of natural waters ranges from 6.0 to 8.5, it was concluded that the probability of sulphonamide hydrolysis in the environment is low. Although hydrolytic degradation of SAs increased with temperature, such high temperatures as 40 °C and 70 °C only occur locally in natural waters. The temperature of natural water is usually between 0 °C and 35 °C, so the hydrolysis rates of SAs established at 20 °C seem to be the most representative. At 20 °C and in acidic solution, the hydrolytic rates of 8 of the 10 SAs after 30 days' degradation were less than 10%. Such conditions prevail in certain soils, especially peaty soils. Moreover, at 20 °C and in neutral solution, the hydrolysis rate of 30-day degraded sulphadiazine rose by only 5%. The long half-lives of sulphadimethoxine, sulphamerazine and sulphadimidine, determined in this study, confirmed their high hydrolytic stabilities in the environment. The results obtained are in agreement with the data recently published by Loftin et al. [27], who investigated the hydrolytic stabilities of sulphachloropyridazine, sulphathiazole and sulphadimethoxine in buffer solutions at pH 5, 7 and 9 at 7 °C, 22 °C and 35 °C, respectively, and sulphathiazole in active sludge chambers at pH 7.4, 7.9 and 6 °C and 10 °C [27,47]. They are dissimilar only for sulphachloropyridazine hydrolysed in neutral solution (those authors found this to be a hydrolytically stable compound), but this hydrolysis was carried out for a shorter time (3 weeks) and at lower temperatures [27].

It should be mentioned that OECD 111 procedure does not take into account such environmental conditions as dissolved oxygen and salinity/electrical conductivity. However, these parameters should not influence the process of SAs hydrolysis because oxygen is not involved in this process and additionally sulphonamides are known to not oxidise. Thus, the presence of oxygen or salinity in environment should not change the fact that hydrolysis of SAs undergo really unwillingly.

4. Conclusions

In this study, the hydrolytic stabilities of 12 sulphonamides were analysed according to the procedure recommended by EMEA and FDA OECD 111. An acidic pH solution was found to be most favourable to hydrolysis, followed by neutral and alkaline solutions. A rise in solution temperature increased SA hydrolysis, but degradation of the SAs was still low. The suggested mechanisms of SA hydrolysis explain why these compounds decompose with difficulty, even though some compounds hydrolyse more readily than others, and why hydrolysis takes place more easily at lower pH. Because SAs are highly stable towards hydrolysis and since they are extensively used in human and animal medicine, these compounds are bound to accumulate in the environment. The hydrolytic stability data presented here conform with the rules required for 1st category quality data for the environmental risk assessment of pharmaceuticals.

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