Synthesis and antibacterial activity of \( N \)-pyridin-2-ylbenzenesulfonamide nanoparticles

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Abstract

Sulfonamide nanoparticles were prepared by reacting benzenesulfonyl chloride with amine groups present in acetonitrile solvent. Ultrasonic treatment was applied for preparation of the sulfonamide nanoparticles. The produced sulfonamide nanoparticles were characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and other techniques. The antibacterial activity of sulfonamide nanoparticles was tested against microorganism and compared with microscale sulfonamide antibacterial properties.

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1. Introduction

In the field of medicine, nanoparticles are being explored extensively because of their size dependent on chemical and physical properties. This makes them an interesting candidate for application in both in vivo and in vitro biomedical research. The result of their integration in the field of medicine has led to their application mainly in targeted drug delivery, imaging, sensing, and artificial implants. Efficient antibacterial activity of medicine nanoparticles was observed against multidrug resistant and highly pathogenic bacteria [1]. Nanoparticles have a very large surface area and much larger particle numbers, thereby exhibiting higher antimicrobial activity [2,3].

Sulfonamides possess many types of biological activities, and representatives of this class of pharmacological agents are widely used in medicine as antibacterial [4], hypoglycemic [5], diuretic [6,7], anti-carbonic anhydrase [6,8] and antithyroid [9]. Sulfonamides have been used as protecting groups of OH or NH functionalities for easy removal under mild conditions [10]. Even though many synthetic methods have been reported [11], the sulfonylation of amines with sulfonyl chlorides in the presence of a base is still being used as the method of choice because of high efficiency and simplicity of the reaction [12]. However, this approach is limited by the formation of undesired disulfonamides with primary amines and by the need of harsh reaction conditions for less nucleophilic amines such as anilines [13]. Therefore, developing a general, mild and novel method in order to synthesize sulfonamides in the absence of a strong base is necessary. In present work, we have introduced an efficient method for the synthesis of sulfonamides via reaction of 2-aminopyridine with benzenesulfonyl chloride in the presence of aprotic solvent at room temperature. The ultrasonic treatment applied for preparation sulfonamide nanoparticles. Sulfonamide was synthesized and characterized by using elemental analyses, FTIR, NMR. Bacterial activities of sulfonyl compounds were studied against gram-positive bacteria: \textit{Staphylococcus aureus}, and gram-negative bacteria: \textit{Escherichia coli} by using minimum inhibitory concentrations (MICs) method.

2. Materials and methods

Amines, sulfonyl chlorides and solvents were purchased from the Merck Company. \textsuperscript{1}H and \textsuperscript{13}C NMR spectra of
deuterated chloroform (CDCl₃) solutions of the compounds were registered on a Bruker WM-300 spectrometer (300 MHz) using tetramethylsilane as internal standard. The infrared spectra of the compounds as KBr-disks were recorded in the range of 400–4000 cm⁻¹ with a Mattson 1000 FT spectrometer. The morphology of synthesized sample was studied using a sputter coating technique with gold as covering contrast material. Melting points of sulfonylamide derivatives were determined with a Gallenkamp melting point apparatus. Powder X-ray diffraction (XRD) was carried out on a Philips diffractometer of X’pert Company with monochromatized Cu-Kα radiation. A multiwave ultrasonic generator (Sonicator-3000; Misonix, Inc., Farmingdale, NY, USA), equipped with a converter/transducer and titanium oscillator (horn), 12.5 mm in diameter, operating at 20 kHz with a maximum power output of 600 W. The modiolution broth method was used to determine the antibacterial and antifungal activities of compounds against the bacteria; gram-positive: *Staphylococcus aureus* ATCC 25923 and gram-negative: *Escherichia coli* ATCC 8739.

### 2.1. Synthesis of sulfonylamide

Acetonitrile (10 ml), benzenesulfonyl chloride (1 mmol) and 2-aminopyridine (1 mmol) were mixed and stirred at room temperature for the appropriate reaction time (Table 1). The obtained precipitate was filtered, washed with THF, recrystallized from ethanol and dried in *vacuo* over P₂O₅. They are colorless and light yellow crystalline solids, stable at normal conditions and soluble in hot methanol and ethanol.

**Table 1**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amines</th>
<th>Benzenesulfonyl chloride</th>
<th>Product</th>
<th>Time (min)</th>
<th>Isolated yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-pyridin-2-ylbenzenesulfonyamide</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td>120</td>
<td>80</td>
</tr>
</tbody>
</table>

N-2-pyridin-2-ylbenzenesulfonyamide spectra data (Table 1)

m.p. 157–160 °C; Anal. cal. for C₁₁H₁₀N₂O₃S (234.05): C, 56.56; H, 4.51; N, 11.89; S, 13.56. Found: C, 56.49; H, 4.33; N, 11.99; S, 13.68%. IR (KBr, cm⁻¹): 3134.19 (w), 3236.44 (w), 3022.61 (w), 2811.77 (w), 2752.6 (w), 1925.12 (w), 1629.69 (s), 1384.24 (m), 1139.51 (s), 1272.1 (s), 779.70 (s), 684.44 (w), 586.56 (s). IR ¹H-NMR (CDCl₃, TMS, 300 MHz): δ (ppm) 10.83 (s, 1H), 6.72–8.37 (m, 9H). ¹³C-NMR (CDCl₃, TMS, 75 MHz): δ (ppm) 108.377, 113.252, 125.867, 128.364, 131.481, 138.251, 148.538, 161.130.

### 2.2. Synthesis of sulfonylamide nanoparticles

The use of ultrasound in chemical reactions in solution provides specific activation based on a physical phenomenon: acoustic cavitation. Cavitation is a process in which mechanical activation destroys the attractive forces of molecules in the liquid phase. The following sonochemical effects can be observed in chemical reactions and processes: increase in reaction speed, increase in reaction output, more efficient energy usage sonochemical methods for switching of reaction pathway, performance improvement of phase transfer catalysts, avoidance of phase transfer catalysts use of crude or technical reagents, activation of metals and solids increase in the reactivity of reagents or catalysts improvement of particle synthesis coating of nanoparticles.

To prepare the sulfonylamide precursor an amount of benzenesulfonyl chloride solution with concentration of 1 M was added to the 1 M solution of amines in acetonitrile. Then the suspension was ultrasonically irradiated with a high-density ultrasonic probe immersed directly into the solution. The obtained suspension was allowed to age for 70 min. The precipitate was separated from mother liquor by using a centrifuge at 4000 rpm for 3 min. The final product was dried at 50 °C in a vacuum system. The working parameters of the ultrasonic device were 60 kHz and 40 W/cm² [14].

### 2.3. Antibacterial activity assays

The standard strains of *S. aureus* and *E. coli* used were obtained from Tehran University of Medical Sciences. The organisms were subcultured in nutrient broth at 37 °C for 6 h prior to antimicrobial testing.

The antimicrobial properties of the sulfonylamides were investigated in the form of the general sensitivity testing and minimum inhibitory concentration (MIC) with respect to freshly cultured targeted organisms. The two considered organisms are a gram positive (*Staphylococcus aureus* ATCC 25923) and a gram negative (*Escherichia coli* ATCC 8739) organisms which are associated with the gastrointestinal tract damage in man and animal.

### 2.4. Antimicrobial screening

The agar diffusion technique as described by Adeniyi and coworkers [15] was used to determine the antimicrobial activity of the synthesized compounds. Broth cultures of the test isolates (0.1 ml) containing 1 × 10⁷ cells/ml of organism were introduced into a sterile Petri dish, added 15 ml of Mueller Hinton agar, thoroughly mixed and allowed to solidify. The seeded plates were allowed to set and wells 6 mm in diameter were made on them using sterile standard cork borer. The wells were filled with 0.4 ml of each sulfonylamide solution in an appropriate solvent at a concentration of 100 µg/ml (0.02 g of sulfonylamide dissolved in 20 ml distilled ethanol). Plates were incubated for
24 h at 37 °C. The assay was conducted at regular intervals of 24 h until a marked decline in the potency of the sulfonamide solution to inhibit the growth of the test organisms was noticed. At the end of the incubation period, the Petri dishes were collected and zones of inhibition that develop were measured in millimeters. The procedure was repeated for the streptomycin (standard).

2.5. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined by Russell and Furr [16]. Different concentrations of the sulfonamides of 50, 25, and 2 µg/ml were used. The agar was streaked with an overnight broth culture of the bacterial strains and incubated overnight. The plates were then examined for the presence or absence of growth. The minimum concentration that completely inhibited macroscopic growth was regarded as the minimum inhibitory concentration, MIC, of sulfonamide. The procedure was repeated for streptomycin (standard). Selectivity index (SI) is the ratio of the zone of the inhibition of the drug to that of the streptomycin. The MIC values of the sulfonamide compounds are presented in Table 3.

3. Results

Fig. 1 shows the XRD patterns of typical samples of sulfonamide prepared by the sonochemical process. Estimated from the Sherrer formula for the calculation of particle sizes from the broadening of the XRD peaks \( D = 0.891\lambda/\beta \cos \theta \), where \( D \) is the average grain size, \( \lambda \) is the X-ray wavelength (0.15405 nm), and \( \theta \) and \( \beta \) are the diffraction angle and full-width at half maximum of an observed peak [17], respectively,
the average size of the particles was found to be around 24 nm. TEM observations indicated that the mean size of particles was about 19 nm (Fig. 2). The difference between the values of particle size obtained by the XRD method from TEM is due to the fact that the former is the measure of entire powder, whereas TEM results correspond to a few milligrams of the sample used. The FTIR spectrum of sulfonamide nanoparticles is shown in Fig. 3. They exhibit the expected absorption bands in the IR. Relatively weak band around 3134.46 cm\(^{-1}\) in \(N\)-pyridin-2-ylbenzenesulfonamide is due to the \(\nu(N-H)\) vibration. Absorption bands with variable intensity in the frequency range \(\nu_S(1358.26)\) and \(\nu_{as}(1383.91)\) cm\(^{-1}\) in sulfonamide correspond to \(\nu(S=O)\) vibrations. \(\nu(S-N)\) vibration is found at 960.17 cm\(^{-1}\) in sulfonamide.

4. Discussions

The sulfonamides are an important class of antibacterial drugs widely used in veterinary medicine for therapeutic purposes [18,19]. The distinctive property of the synthesized sulfonamide with the use of acetonitrile solvent is that they...
Table 2
In vitro antibacterial activity of sulfonamide and nano sulfonamide. The more effective the drug, the greater the zone of inhibition, ZOI (in mm). Selective index, SI, is ZOI of sulfonamide or nano sulfonamide divided by ZOI of streptomycin as clinical reference.

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th></th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ZOI (mm)</td>
<td>SI</td>
<td>ZOI (mm)</td>
</tr>
<tr>
<td>Sulfonamide</td>
<td>16</td>
<td>1.23</td>
<td>25</td>
</tr>
<tr>
<td>Nano sulfonamide</td>
<td>25</td>
<td>1.90</td>
<td>38</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
The minimum inhibitory concentration (µg/ml), MIC, values of sulfonamide and nano sulfonamide.

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th></th>
<th>S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamide</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Nano sulfonamide</td>
<td>48</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

could be easily prepared with a high degree of purity, and because of their non-greasy property the product could be easily converted into nanoparticles. The biological relevance of the synthesized sulfonamide was authenticated by screening them in vitro against Staphylococcus aureus and Escherichia coli. Sulfonamides and nano sulfonamides showed low activities on E. coli. The zones of inhibition (in mm) for sulfonamide nanoparticles against Staphylococcus aureus and Escherichia coli were significantly larger than those for microscale sulfonamide particles (Table 2). The comparative study of effect of the sulfonamides and streptomycin on E. coli and S. aureus could be clearly understood by observing the selectivity index, SI, (Table 2). Both sulfonamides and nano sulfonamides have selectivity indices ranging from 1.23 for sulfonamide to 2.92 for nano sulfonamide. This implies that streptomycin (SI = 1) was less active than the sulfonamide nanoparticles.

Results of the minimum inhibitory concentration (MIC) are summarized in Table 3. The higher MIC values for nano sulfonamide indicate that sulfonamide nanoparticles inhibit E. coli and S. aureus much more than microscale sulfonamide. The obtained results show that sulfonamide nanoparticles inhibited microbial activity much more than the sulfonamide microscale particles, indicating that a smaller dose of the nanoparticles is sufficient to inhibit the growth of the bacteria.

The size of the solid particle influences the solubility since as a particle becomes smaller (24 nm), the effective surface area of the particles increases. Increasing the solubility of sulfonamide nanoparticle increases its inhibition efficiency and reduces its bad side effects.

When sulfonamides are transformed into nano forms the proportion of surface to volume will be increased. The increase in the surface of particles decreases the surface pressure and causes the distant change among the particles or even the distance among the atoms of particles. The change in the distance among atoms of particles and high proportion of surface to volume in nanoparticles will have the same impact on the properties of substances. Therefore, the synthesized nanoparticles of sulfonamides have the capability of being more antibacterial in comparison to their microscale particles.

5. Conclusion

In this study we have reported the synthesis of new sulfonamide derivatives and nano sulfonamide. The structural characterizations of synthesized compounds were made by using the elemental analysis, spectroscopic methods, X-ray diffraction and transmission electron microscope. Sulfonamide and nano sulfonamide show varying degrees of inhibition effects on the growth of the bacteria. The results of sulfonamide nanoparticles have antibacterial activities more than microscale particles.

References