Spectral determination of novel methacryloyl guanidine compounds in water solutions

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Abstract. For production and application of novel biocidal guanidine polymer compounds techniques for monitoring the toxic monomer and oligomer components in polymer aqueous solutions are required. A direct spectroscopic method is offered for determination of methacryloyl guanidine salts, polymer and copolymer in water solutions at native pH. The impact of unsubstituted guanidine and methacrylate guanidine contaminants is also discussed. The spectroscopic method offered is simple, rapid and effective for monitoring contamination of polymethacryloylguanidines with toxic monomer components during synthesis of polymers and copolymers from different monomer salts, during dialysis and other manufacturing stages of biocidal polymers.

1. Introduction

Synthesis of novel guanidine compounds (both macromolecular and low molecular weight) is of particular importance due to them being highly potent in biocide and antiseptic applications [1–3]. Guanidine polymers possess general biocidal properties versus a wide spectrum of both gram-positive and gram-negative bacteria [4–6], and are widely used as a scaffold in the design of specific bioactive compounds with low cytotoxicity and, therefore, human- and livestock-safe [6].

During the last 10 years a series of novel substituted guanidine compounds were synthesized TIPS RAS, their high biocidal activity proven both individually and as a part of polymer composite materials [5, 7]. Substituted guanidines, particularly methacryloyl-guanidines, surpass unsubstituted guanidine in biocidal activity [8, 9]. Due to promising prospects of their practical usage, the question of their determination and identification in solutions is indispensable during industrial and commercial introduction. As the toxicity of biocidal guanidine compounds varies profoundly with their molecular weight (monomers are generally more toxic than polymers), it is essential to develop techniques for monitoring and controlling the low molecular weight components in polymer products concurrently with their synthesis.

Usually spectral determination of guanidine compounds is carried out via IR-range guanidine group characteristic bands, which do not change on polymerization [10]; for methacryloylguanidines, double bond opening on polymerization is clearly and quantitatively identifiable [5]. In UV-vis spectroscopy, guanidine attenuation happens in near UV range (200–300 nm) [11]. Thus, for quantitative determination of guanidines and guanidine groups in arginine, colored complex formation with naphthol is usually employed (Sakaguchi reaction, [12]), which is a finicky sophisticated technique and due to some circumstances [13] is unfit for determination of guanidine monomers in the presence of their polymers in aqueous solutions.

For PHMG, a colored complex of polymer with Eosin Y at pH 2.5, absorbing at 540 nm, is employed for its determination in water [14]. The latter is proposed for creating samplers for detecting PHMG aerosol presence in air [15]. Solution separation could be achieved in general case by
electrophoresis, which was proven for mixtures of PHMG, its monomer and guanidine hydrochloride (GHC) [16], and is potentially applicable for novel substances.

Some substituted guanidines demonstrate a red shift in their attenuation bands, allowing for direct quantitative determination in mid UV. Ab initio quantum chemical calculations prove the red shifts to be connected with both increase in the size of conjugated system and ionized complex formation of guanidinium cation with organic counterion. Guanidinium cation π-π* transition band lies at about 160 nm [17], while for phenilguanidinium and 1-naphthylguaninium at 190 and 220 nm, accordingly [18]. Complex formation shifts the n-π* transition band; phenilguanidinium maximum in the presence of complexing counterions is shifted from 210 nm to up to 200 nm (230 nm, F−) [18]. In view of the above, methacryloylguanidines in aqueous solution should have attenuation bands in the UV-vis range, sensitive to their degree of polymerization and monomer counterions.

The aim of this article is to obtain and analyze UV-vis spectra of novel methacryloyl substituted guanidine compounds in the 190–250 nm range. Special attention is paid to mixed aqueous solutions, at natural pH typical for their synthesis and manufacturing. We believe these results to be useful for analytical and express monitoring methods in production of guanidine-based biocidal additives.

2. Materials and methods
The objects of study were novel methacryloyl substituted guanidine compounds (table 1, table 2), synthesized and characterized at TIPS RAS. Polymers were fully separated and purified by filtering the reaction mass, followed by precipitation into acetone, multiple leaching with same, and dialysis on “Spectrapor MWCO 3500” membranes to remove <3000 Da fraction. Products’ structure and composition were verified by 1H NMR spectroscopy immediately afterwards. The syntheses are described in detail in [5, 19].

| Compound (designation) | Chemical formula | MW (g/mol)\textsuperscript{a} | pH\textsuperscript{b} |
|------------------------|------------------|-----------------------------|------------------|
| Guanidine hydrochloride (GHC) | \[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2 \\
\text{Cl}\,\text{(aq)}
\end{array}
\] | 95.5 | 6.4 |
| Methacrylate guanidine (MAG) | \[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2 \\
\text{O}
\end{array}
\] | 145.2 | 6.2 |
| Methacryloylguanidine hydrochloride (MGHC) | \[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2 \\
\text{O}
\end{array}
\] | 163.6 | 6.1 |
| Methacryloylguanidine trifluoroacetate (MGTF) | \[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2 \\
\text{CF}_3\text{COO}\,\text{(aq)}
\end{array}
\] | 241.2 | 5.6 |
| Methacryloylguanidine acetate (MGAc) | \[
\begin{array}{c}
\text{H}_2\text{N} \\
\text{NH}_2 \\
\text{CH}_3\text{COO}\,\text{(aq)}
\end{array}
\] | 187.2 | 6.1 |

\textsuperscript{a} molecular weight
\textsuperscript{b} pH of 1.14 mmol/l aqueous solution at 20°C

Preconditioned in vacuo at 34 °C for 50 hours, the solid powder samples were kept in a dessicator due to their hygroscopic behavior. Fresh solutions of monomers and polymer with concentration 2.85±0.05 mmol/l were prepared and diluted to working concentrations with bidistilled water using standard analytical techniques. Measurements were carried out on a split-beam Specord M40 spectrometer (Germany), in 2 cm quartz cells at 20±1 °C in the range of 188–1100 nm to a precision of 0.001. The total concentration of mixed solutions was equalized at 30 μmol/l concentration by the
absorption in right isosbestic point (233 nm). pH was measured at 20±1 °C on an ANION 7000 pH-meter (Russia) with a glass–silver electrode pair.

Table 2. High molecular weight guanidine compounds studied.

| Compound (designation)                        | Chemical formula | pH  |
|----------------------------------------------|------------------|-----|
| Polymer of MAG (PolyMAG)                    |                  |     |
| Polymer of MGHC (PolyMGHC)                  |                  |     |
| Copolymer of MGHC with diallyldimethylammonium chloride 78:22 mol. % (coPolyMGHC) |                  |     |

This pH of 1.14 mmol/l aqueous solution at 20°C

3. Results and discussion

The spectra of low-molecular weight guanidine compounds are presented on figure 1.

Figure 1. Electronic absorption spectra of low molecular weight guanidine compounds in bidistilled water at natural pH:
1 — GHC;
2 — MGHC;
3 — MGTFA;
4 — MGAc;
concentration 30 μmol/l.

GHC solutions a show an absorption maximum at 190 nm with $\varepsilon = 7.6 \times 10^3$ l/mol·cm (figure 1, curve 1). In aqueous solutions GHC exists as a guanidinium cation (pKa=13.5 [11]) with absorption band in the far UV range; the detected maximum lies at the shoulder of the absorption band and at the boundary of the measurement range, so the intensity dependence is linear only at concentrations of 16–30 μmol/l. Furthermore, in aqua guanidine is prone to oxidizing to urea (and subsequently to ammonium carbonate) with the same band position and $\varepsilon \approx 1 \times 10^3$ l/mol·cm [20], leading to a decrease in measured concentration compared to freshly prepared solutions. This also hinders the direct quantitative measurement of GHC in aqueous solution.

A direct spectropolarimetric technique is described for measuring unsubstituted GHC and urea in dialyzed aqueous protein solutions [21], where the first hindrance is curcumvented by measuring the attenuation at the inflection point (in the range 190–230 nm), allowing for quantitative determination in concentrations from 6 μmol/l in distilled water and from 120 μmol/l in phosphate buffer.
Nevertheless, applying the same treatment to our spectra did not enhance neither the limit of detection nor the precision of measurement.

The introduction of auxochromic methacryloyl group into guanidinium cation leads to a red shift of attenuation band to a maximum of 210 nm (figure 1, curves 2–4). Molar extinction coefficients are similar for MGHC and MGTFA, and is slightly lower for MGAc. As the cation structure for those compounds is the same, it can be accounted for by the difference in counterions: MGHC and MGTFA are strong acid salts ($pK_a = -10$ and $pK_a = 0.23$, correspondingly), while MGAc is the salt of weak acetic acid ($pK_a = 4.76$). Neither GHC nor MGHC in these concentrations shift the absorption maxima of anionic dye Fast Green FCF, which forms complexes with arginine moieties in proteins.

The polymerization of MGHC with $\text{C}=$ bond of methacryloyl group being opened results in $\pi$-$\pi^*$ band shifting to a 201 nm position in polymer (figure 2, curve 2).

Figure 2. Electronic absorption spectra of methacryloylguanidine compounds in bidistilled water at natural pH:
1 — MGHC;
2 — PolyMGHC;
3 — coPolyMGHC;
concentration 30 μmol/l.

PolyMGHC quantitative measurement is possible both via 201 nm band and via n-$\pi^*$ band at 238 nm, or at the saddle point (223 nm). For both PolyMGHC and coPolyMGHC, the maximum at 238 nm coincides with the right isosbestic point at 238 nm (figure 2). As neither diallyldimethylammonium, nor its oligomer blocks have an absorption band at $\lambda>$200 nm [22], it is possible to recalculate the attenuance of coPolyMGHC to MGHC monomer equivalent for similar determination in mixed monomer/copolymer solution.

CoPolyMGHC spectra have the same band positions as PolyMGHC (201 and 238 nm), but their relative intensity is different; furthermore, for solutions diluted to less than 10 μmol/l, the relative intensities also change, compared to higher concentrations. This could be attributed to different methacryloyl block conformation depending on polymer chain structure in solution, and by general supramolecular structure differences; the implication to spectral measurement is that for those concentrations, the 238 nm band is better suitable.

No attenuance was detected which could be attributed to colloidal scattering in those conditions and concentrations; additional care should be taken, however, on measurements in higher ionic strength solutions and at higher polymer concentrations.

These factors allow for quantitative determination of isolated MGHC, PolyMGHC and coPolyMHC in aqueous solutions (figure 3).

Band characteristics, ranges of detection and linear regression parameters are presented in table 3. For mixed solutions, the use of absorption at the right shoulder of 238 nm band or a total peak decomposition is necessitated for polymer, and the 223 nm for monomer, due to close proximity of absorption bands and their high half-width (25–30 nm), and a non-additive increase in absorption in sub-205 nm range.

Thus, direct spectrophotometry allows for effective determination of methacryloylguanidine monomers, polymer, and copolymer in unbuffered aqueous solution at natural pH without prior component separation, but lacks in GHC contaminant detection. The spectral measurement range
rules out the usage of default glass or plastic transmission cells, and a split-beam geometry for the spectrometer is preferred to minimize the water attenuation.

**Figure 3.** Calibration curves:
1 — GHC;
2 — MGHC;
3 — MGTFA;
4 — MGAc;
5 — PolyMGHC (238 nm);
6 — coPolyMGHC (238 nm).
Measurement uncertainty is within the size of symbols.

**Table 3.** Attenuation bands and molar extinction coefficients of guanidine compounds.

| Compound   | λ_{max} (nm) | ε·10^{-3} (l/mol·cm) | Linear range of detection (μmol/l) | Linear regression A=k·C parameters |
|------------|--------------|----------------------|-----------------------------------|-------------------------------------|
| GHC        | 190          | 7.6±0.2              | 16–30                             | 15.2±0.2                           |
| MGHC       | 212          | 13.1±0.1             | 4–80                              | 26.2±0.2                           |
| MGTFA      | 212          | 14.3±0.1             | 4–80                              | 28.2±0.2                           |
| MGAc       | 210          | 8.5±0.1              | 4–80                              | 16.6±0.2                           |
| PolyMGHC   | 201          | 13.5±0.3             | 4–40                              | 27.0±0.2                           |
| coPolyMGHC | 238          | 3.6±0.1              | 4–80                              | 6.9±0.2                            |
|            | 201          | 8.9±0.1              | 4–90                              | 17.7±0.3                           |

| Compound   | k·10^{-3} (l/mol) | Regression coefficient r² |
|------------|-------------------|---------------------------|
| GHC        | 15.2±0.2          | 0.999                     |
| MGHC       | 26.2±0.2          | 0.998                     |
| MGTFA      | 28.2±0.2          | 0.999                     |
| MGAc       | 16.6±0.2          | 0.999                     |
| PolyMGHC   | 27.0±0.2          | 0.985                     |
| coPolyMGHC | 17.7±0.3          | 0.999                     |

4 For all substances studied, the limit of determination is sufficiently low (<1 μmol/l)

One additional contaminant to consider is methacrylate guanidine, which is a byproduct of methacroyloyl guanidine synthesis, capable of polymerization in the same condition as methacroyloylguanidine. To estimate the possibility of analytical monitoring this contaminant in MGHC/PolyMGHC aqueous solutions, additional spectra were taken (figure 4).

**Figure 4.** Electronic absorption spectra of guanidine compounds and contaminants in bidistilled water at natural pH:
1 — GHC;
2 — MAG;
3 — PolyMAG;
4 — methacrylic acid;
5 — difference spectrum of MAG and GHC; concentration 30 μmol/l.
On methacrylate guanidine dissociation in water, unsubstituted guanidinium cation and methacrylic acid anion are produced. MAG solution spectrum amounts to a superposition of guanidinium and methacrylate ion attenuation bands (figure 4, curves 1, 2, 5), as the band positions in difference spectrum (figure 4, curve 5) are identical to those in the spectrum of methacrylate anion (figure 4, curve 4), and the difference in intensity of extinction is explained by methacrylic acid partial dissociation in measurement conditions (pKa = 4.46). On polymerization of MAG the methacrylic group double bond opens, so the aqueous solution of PolyMAG (curve 3) has the same spectrum as guanidinium.

MAG attenuation bands are essentially non-linear vs concentration, and so is the case for pure methacrylic acid without guanidinium cations [23]). Furthermore, both methacrylic acid and its salts are unstable in aqueous solution and the bands coincide with the bands of other components and contaminants. Thus, they could not be used for direct quantitative determination, and monitoring MAG in the reaction mixture or product should be done in other ways.

4. Conclusion
Novel monomer and polymer methacryloylguanidine compounds were spectroscopically characterized for the first time in the UV-vis range. Attenuation bands and molar extinction coefficients are determined for them in aqueous solutions, allowing for quantitative determination in 4–80 μmol/l range. UV-vis spectroscopy is effective for monitoring contamination of polymethacryloylguanidines with monomers during polymer and copolymer synthesis from different monomer salts at natural pH, dialysis and other biocidal polymer manufacturing stages.

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