

EXPERIMENTAL STUDY

Iodine in drinking water in Denmark is bound in humic substances

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Abstract

Objective: The iodine intake level is important for the occurrence of thyroid disorders in a population. We have previously found that iodine in drinking water is related to iodine excretion but whether iodine is present as iodide or bound in other molecules remains unknown.

Design: We measured iodine in drinking water from 22 locations in Denmark. Six locations were selected by iodine content for further tap water analysis (Skagen 140 µg/l, Samsøe 56 µg/l, Nykøbing S. 50 µg/l, Nakskov 40 µg/l, Ringsted 38 µg/l, Copenhagen 19 µg/l).

Methods: HPLC size exclusion before (Skagen) and after (all sites) freeze drying and measurement of absorbance (280 nm) and iodine in fractions, and fluorescence spectroscopy of bulk organic matter in Skagen drinking water.

Results: Iodine content was unaltered after 3 years ($P = 0.2$). All samples contained organic molecules with characteristics similar to humic substances. Most iodine eluted with humic substances (Skagen 99%, Ringsted 98%, Nykøbing S. 90%, Copenhagen 90%, Samsøe 75%, Nakskov 40%). Changing pH and ionic strength and preincubation with iodide indicated that iodine was bound in humic substances. Humic substances may affect thyroid function but differ with geology. Geological and geochemical data agree with tap water humic substances having been released from marine deposits. Iodine is abundant in the marine environment and marine deposits are particularly rich in iodine. Correlation analysis ($r = 0.85$, $P = 0.03$) conform to iodine in drinking water, suggesting marine humic substances at the source rock.

Conclusion: Iodine in Danish drinking water varied considerably. In drinking water with a high iodine content, the iodine mainly eluted with humic substances derived from marine source rock. We hypothesize that iodine in drinking water in general suggests coexisting humic substances of marine origin.

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Introduction

The iodine intake level is important for the occurrence of thyroid disorders in a population, and both high and low iodine intake levels are associated with increased risk of disease (1, 2).

Dietary iodine content is decisive for the intake of iodine (3). Water is an ubiquitous component of the diet and ground water is an important drinking water resource in many countries. We have previously shown that the content of iodine in tap water exhibits major regional differences in Denmark (4) where more than 99% of drinking water is ground water. These differences are associated with differences in population iodine intake levels (4, 5) and with differences in the incidence and prevalence of thyroid disorders (6).

Tap water iodine content has been studied in many parts of the world (4, 7–12). It has been associated with thyroid disorders (7–12) and subsurface geology (10–12). However, data on whether iodine in drinking water is present as iodide anions or not are lacking (13).

Chemical analysis of natural waters has demonstrated variable amounts of organic matter. The majority of organic matter is aquatic humic substances (14–20). Humic substances are heterogeneous mixtures of organic molecules produced by decomposition of plant and animal tissues (15–17). The concentration and composition of humic substances in ground water vary between aquifers, depending on the geology and chemistry of the aquifer (16–18). Some humic substances may affect thyroid gland morphology and function when included in the diet (21–23). This occurs because humic substances complex

with or convert into antithyroid and goitrogenic compounds during degradation in the gut (21, 23).

The aim of our investigation was to elucidate the chemical nature of the iodine in drinking water in Denmark. Interestingly, we found that iodine was part of the humic substances.

Materials and methods

Water samples

Tap water samples were collected from 22 waterworks in Denmark for determination of iodine content (Fig. 1). The waterworks were chosen to verify low and high drinking water iodine contents compared with our previous investigation (4). Six waterworks were selected for further tap water analysis: one location with a high iodine content (three to four times sea water level) and five locations with a medium-high iodine content in tap water (approximately sea water level) (Fig. 1). Tap water was processed groundwater from five locations and, in Copenhagen, ground water mixed with up to 2% surface water. Samples were collected on 3 separate days from all locations. Furthermore, Skagen tap water was collected at 2-month intervals for 6 months, and once every year for 4 consecutive years.

At five waterworks, ground water had been treated with aeration, sedimentation and filtration. Skagen waterworks perform additional chemical coagulation before sedimentation, adsorption in granular-activated carbon contractors and tandem sand filtration. In Skagen, with the most extensive water treatment, the iodine content before water treatment (ground water) was $152.7 \mu\text{g/l}$ ($\pm 4.0 \mu\text{g/l}$) and after water treatment it was $139.7 \mu\text{g/l}$ ($\pm 5.2 \mu\text{g/l}$). Thus, water treatment reduced the iodine content of Skagen water by approximately 8.5%.

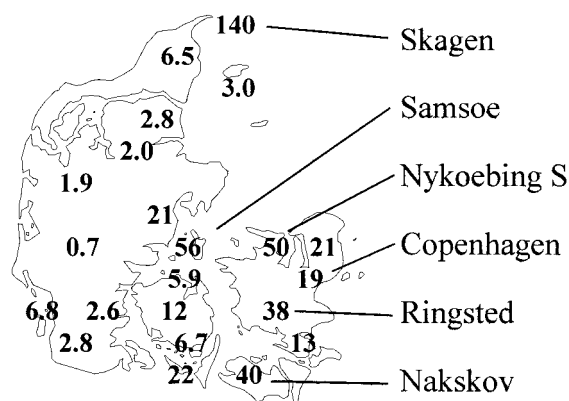


Figure 1 Map showing the iodine content ($\mu\text{g/l}$) of tap water collected at 22 waterworks in Denmark. Tap water from the six sites indicated were analysed further in this study.

Procedures and solutions

Tap water samples were collected in iodine-free polyethylene containers from the final tap before leaving the waterworks. Samples were kept in the dark at 4°C until analysis. Freeze-dried samples from the six selected waterworks were stored in an oxygen-free environment until they were redissolved at 1:10 in ultrapure water from a Mili-Q water purification system (Millipore, Bedford, MA, USA) for further analysis.

High performance liquid chromatography (HPLC) size exclusion was performed on an ÄKTA purifier (Amersham Pharmacia Biotech, Freiburg, Germany) using a Superose 12 HR 10/30 column (Amersham Pharmacia Biotech). This is an agarose gel with exclusion limits from 1000 to 300 000 Da (limits stated by supplier), a void volume (V_0) of 8.4 ml (permeation coefficient $K_{av} = 0$) and a total permeation volume (V_{total}) of 25.0 ml ($K_{av} = 1$) (determined for the column using dextran blue and glucose). The column was calibrated with globular proteins (LMW Calibration kit; Amersham Pharmacia Biotech) for molecular weight determinations according to the log-linear method (24) and the relation was molecular weight = $e^{16.8 - (0.423 \times \text{V elution})}$. Five hundred microlitres of raw or resuspended tap water were added to the column after filtering through a $0.20 \mu\text{m}$ membrane (Minisart; Sartorius, Göttingen, Germany) to eliminate particulate matter. Tris buffer (10 mM, pH 7.0) was the eluent in the main experiment. Elution speed was 1 ml/min and pressure was 1.45–1.52 MPa. Absorbance at 280 nm was registered and effluent was collected in fractions of 1.5 ml. Experiments were carried out at 21°C and performed in triplicate.

Findings were validated by repeated procedures on Skagen tap water at changing pH values (at 10.0, 9.0, 8.0 and 7.0 using a 10 mM Tris buffer, at pH 6.0 and 5.0 using a 10 mM phosphate buffer, and at pH 4.0 using a 10 mM citric acid buffer). Also HPLC size exclusion was performed on Skagen tap water at increased ionic strength (100 mM Tris buffer, pH 7.0). Furthermore, the fraction of Skagen tap water containing bulk organic substance (fraction 9) was investigated by emission fluorescence spectroscopy (PTI Fluorescence System 1.4; Photon Technology International, Lawrenceville, NJ, USA). It was excited at 280 nm and an emission spectrum was recorded in the range from 300 to 550 nm.

To evaluate the effect of freeze drying on the macromolecules, HPLC size exclusion was performed on Skagen tap water with no pretreatment, after freezing and thawing, and after freeze drying and resuspending at 1:10. The reproducibility of the chromatograms was assessed by HPLC size exclusion on water collected on three different occasions from all sites.

Identical iodine concentrations were seen before and after filtering tap water through a $0.20 \mu\text{m}$ membrane. Also, freeze drying showed no effect on the iodine

concentration in tap water from any of the towns investigated when redissolved 1:1 in Mili-Q water (recovery 90–103%; average 98%).

Twenty millilitres of Skagen tap water (containing 2.8 µg iodine) were mixed with 1 ml potassium iodide 100 mg/l solution (Merck, Darmstadt, Germany) diluted to 3.0 mg/l with MiliQ water (1 ml contained 3 µg iodide). After incubation at 21 °C for 24 h, HPLC size exclusion and determination of iodine in fractions were performed.

Iodine determination

Iodine was determined by the Sandell–Kolthoff reaction modified after Wilson & van Zyl (25) as described previously (26). The principle is evaporation and alkaline ashing of the sample, followed by resuspension and measurement of iodine by the spectrophotometric detection of the catalytic role of iodine in the reduction of ceric ammonium sulfate in the presence of arsenious acid. For determination of iodine content, a 1.5 ml sample was used giving an analytical sensitivity of

2.0 µg/l. The intra-assay coefficients of variation were 9.2% (interval 2–4 µg/l, $n = 8$), 8.7% (interval 5–9 µg/l, $n = 4$), 4.2% (interval 10–15 µg/l, $n = 4$) and 1.5% (interval 15–50 µg/l, $n = 5$). Recovery of added iodine was >95% and not corrected for.

Statistical analysis

Tap water iodine content was compared with results from the previous study (4) by t -test for $[I]_{1998} - [I]_{1995} \neq 0$ as this difference followed the normal distribution. The relation between concentrations of iodine and humic substances in tap water was estimated by Spearman's rho test. A P value of less than 0.05 was considered significant.

Results

Figure 1 shows the iodine content of drinking water collected at 22 waterworks in Denmark. The iodine concentrations in tap water at the individual sites were similar to the results of our previous study (4)

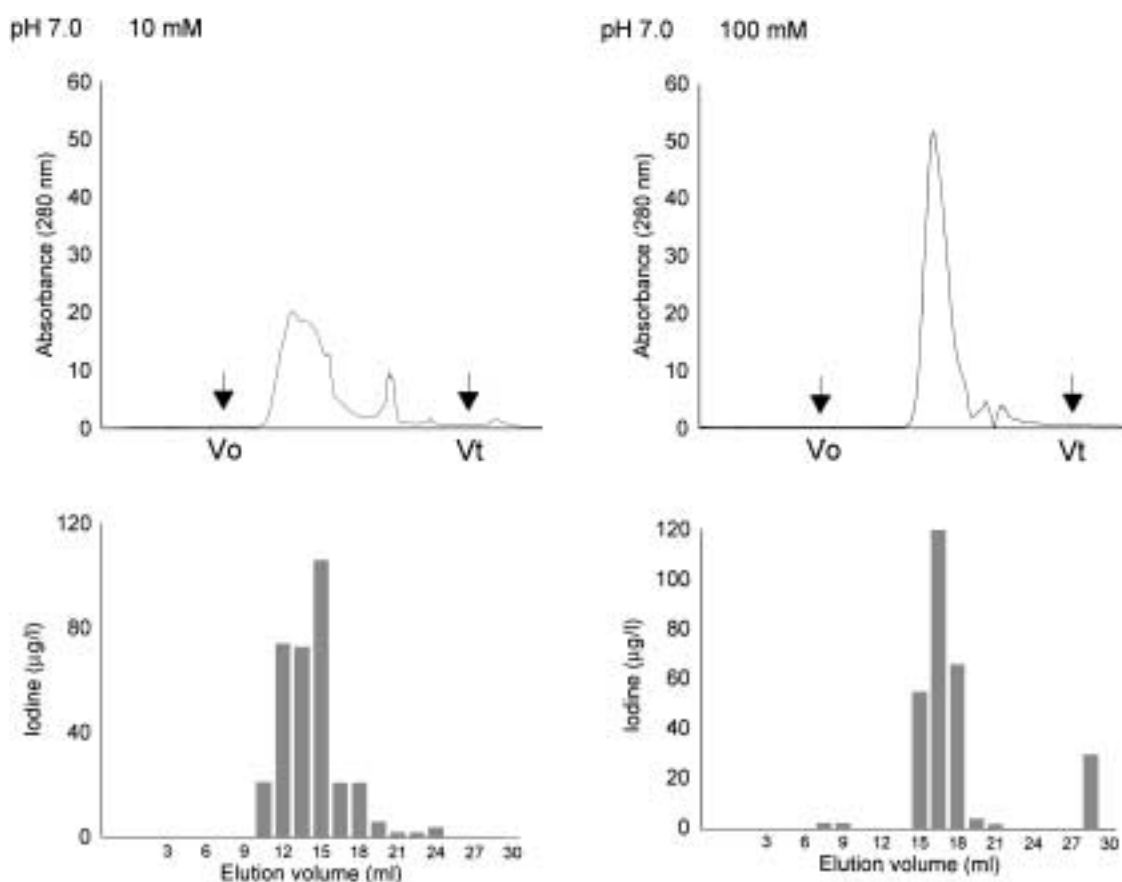


Figure 2 HPLC size exclusion performed on preconcentrated Skagen drinking water at pH 7.0. Absorbance at 280 nm was registered. An increase in ionic strength from 10 mM to 100 mM caused a change in elution of humic substances from K_{av} 0.34 to 0.73 (upper panels) accompanied by an identical change in the elution of most iodine (lower panels). V_t , total volume.

($P = 0.2$). The highest iodine concentration was found in tap water from Skagen. HPLC size exclusion of Skagen tap water without pretreatment showed that iodine eluted corresponding to organic matter (data not shown).

Figure 2 shows the elution of organic matter (absorbance at 280 nm) and iodine in pre-concentrated Skagen tap water. A broad monomodal elution pattern with subtle shoulders and subpeaks was found. At pH 7.0 and ionic strength 10 mM, the main peak in absorbance eluted at approximately V_{elution} 14 ml (K_{av} 0.34). Iodine eluted corresponding to this peak. Increased ionic strength from 10 mM to 100 mM caused a change in elution of organic matter from K_{av} 0.34 to 0.73 (molecular weight 50–3.5 kDa) (Fig. 2, upper panels). This was accompanied by an identical change in the elution of most iodine (Fig. 2, lower panels) while approximately 10% of the iodine eluted as low molecular weight substances (around V_{total}). Figure 3 shows the elution of organic matter and iodine in Skagen tap water at different pH values. Organic matter and iodine eluted simultaneously, and changing the pH caused a parallel change in the elution of organic matter and iodine, demonstrating that iodine in Skagen tap water was part of the organic matter.

An emission fluorescence spectrum of the fraction containing bulk organic matter from Skagen drinking water is depicted in Fig. 4. Apart from the narrow Raman scatter of water at 310 nm, the fluorescence spectrum showed a single broad band peaking at 460 nm. The humic substances are probably highly inhomogeneous. Thus, the fluorescence emission spectrum should be perceived as an average fluorescent signature of the humic substances. Interestingly, there was no trace of the endogenous chromophore, tryptophan, in the spectrum. However, the spectrum was

similar to spectra generally obtained from humic substances (16).

After preincubation of Skagen tap water, the iodide added eluted corresponding to V_{total} (Fig. 5). The iodine content was unaltered in fractions collected corresponding to the elution of humic substances.

Figure 6 shows HPLC size exclusion chromatograms of pre-concentrated tap water samples from the six locations investigated further. A main peak 1 eluted at approximately V_{elution} 14 ml (K_{av} 0.34 at pH 7.0 and ionic strength 10 mM) in drinking water from all locations. This corresponded to a molecular size of about 50 kDa. As can be seen in Fig. 6 the quantity of iodine eluted corresponding to this peak (Skagen 99%, Ringsted 98%, Nykøbing S. 90%, Copenhagen 90%, Samsøe 75%, Nakskov 40%). In Samsøe tap water 25% and in Nakskov tap water 60% of iodine eluted corresponding to low molecular weight substances (around V_{total}).

Figure 7 illustrates the association between concentrations of iodine and humic substance in tap water from six locations. A significant correlation was seen ($r = 0.85$, $P = 0.03$).

Freeze drying caused a narrow absorbance peak 2 to appear at V_{elution} 19.5 ml equal to K_{av} 0.67, corresponding to molecular weight 5 kDa (compare Figs 5 and 2, upper left). Additional changes in the chromatographic patterns obtained were small. HPLC size exclusion chromatograms were identical for tap water samples collected on 3 consecutive days at each site, and collected at Skagen every second month for 6 months and 4 consecutive years (data not shown).

Discussion

We have previously shown that the iodine content of drinking water is important for the iodine intake level of the population in Denmark (4). In the present

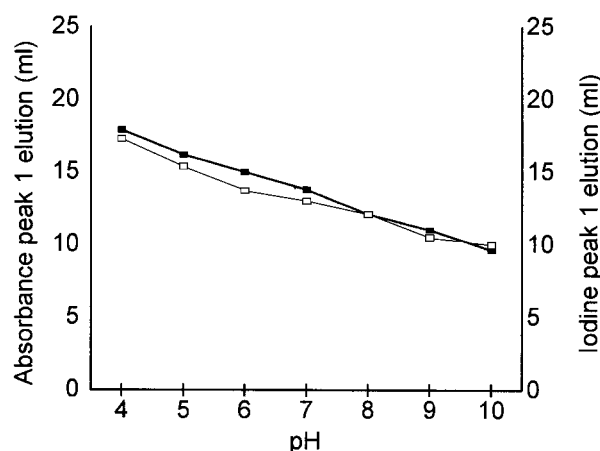


Figure 3 HPLC size exclusion of Skagen drinking water at different pH values. Humic substances (solid line) and iodine (broken line) eluted simultaneously, and changing the pH caused a parallel change in the elution.

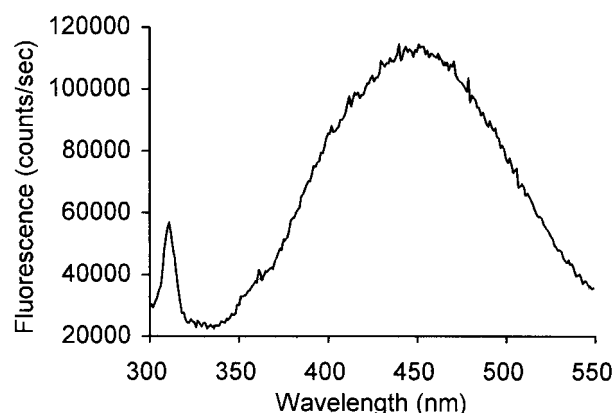


Figure 4 Emission fluorescence spectrum of organic matter in drinking water from the Skagen aquifer. Excited with monochromatic light at 280 nm while an emission spectrum in the range from 300 to 550 nm was recorded.

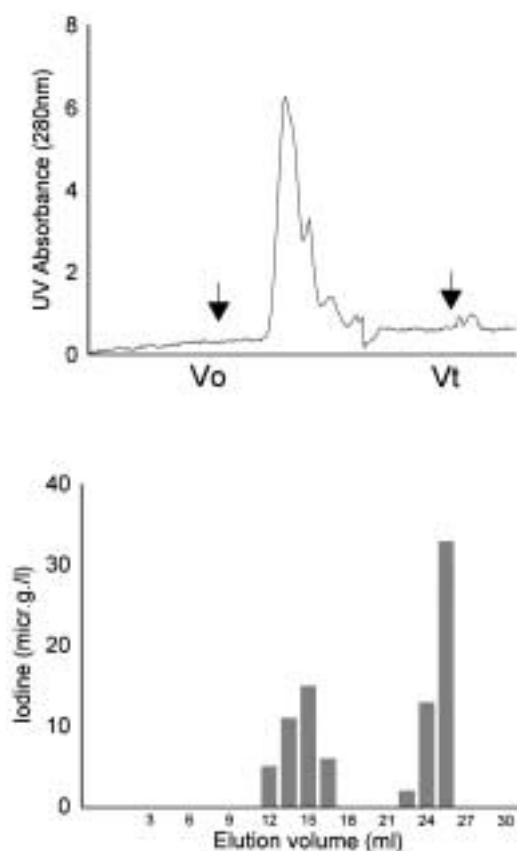


Figure 5 HPLC size exclusion elution pattern of Skagen drinking water (20 ml containing $2.8 \mu\text{g}$ iodine) preincubated for 24 h with potassium iodide (1 ml containing $3.0 \mu\text{g}$ iodide). The iodide added eluted corresponding to V_{total} (V_t).

study, we found that the iodine content of drinking water was unaltered after 3 years and present not as iodide but mostly bound in humic substances.

Skagen tap water iodine content was about nine times higher than the average iodine content at the remaining 21 sites in Denmark ($16 \mu\text{g/l}$). HPLC size exclusion of untreated Skagen drinking water suggested that iodine did not exist as free iodide. This motivated further investigations.

Due to dilution during size exclusion chromatography, samples had to be preconcentrated. Freeze drying provided gentle and sufficient preconcentration of organic matter in drinking water (27) and size exclusion chromatography on a single column gave information on both molecular weight and quantity (14, 20, 28, 29). Using these techniques, we found dissolved organic matter in the drinking water from all six locations investigated, with the highest content in Skagen drinking water.

The HPLC elution pattern of organic matter in our study corresponded to humic substances (14, 17, 28, 29). Humic substances are heterogeneous mixtures of naturally occurring organic molecules. They are produced in sediments in the decay process of plant

and animal tissues as an intermediate step in the eogenetic pathway from higher plant material to kerosene and eventually coal (16, 20). Humic substances are divided into humic acids and fulvic acids based on differences in elemental characteristics and chemical properties (16–19, 30). Recently, humic substances from the Skagen aquifer were characterized in detail (18, 30). The analyses included size exclusion chromatography, fluorescence spectroscopy, nuclear magnetic resonance spectroscopy, determination of dissolved organic carbon, amino acid and elemental composition, and isotope analyses. These investigations concluded that Skagen ground water contains fulvic acids released from 4500-year-old marine deposits (18).

We performed size exclusion chromatography and fluorescence spectroscopy of the bulk humic substances in Skagen drinking water. Our spectroscopic finding was similar to spectra generally obtained in humic substances (16) and similar to those described for fulvic acids from the Skagen aquifer (18). Size exclusion chromatography revealed different absorbances but identical retention volumes between sites. This indicated different concentrations of similar sized humic substances in tap water from all sites investigated. The unaltered findings over the 4 years of repeated sampling demonstrated the permanence and stability of iodine-containing humic substances in drinking water.

The simultaneous elution of humic substances and iodine could be coincidental. Humic substances aggregate in solution depending on pH, ionic strength and concentration, causing variations in molecular size (14, 20, 29). We validated our findings by repeated experiments on Skagen tap water at changing pH and ionic strength with unaltered concentration, and by preincubation with iodine. In all conditions, humic substances and iodine eluted simultaneously and with a constant ratio of iodine to humic substances. Also, the added iodine eluted around V_{total} with an unaltered iodine/humic substances ratio. These experiments imply that iodine is part of humic substances and could suggest that the iodine was covalently bound.

A significant correlation was found between concentrations of iodine and humic substances in drinking water from the different sites investigated. However, in tap water from two locations, some of the iodine (25% and 60%) eluted corresponding to low molecular weight substances. The present investigations revealed no explanation for this difference as no iodine-containing substances were used and no difference in subsurface geology or water treatment was identified.

Marine deposits may be rich in iodine because iodine is assimilated and organically bound in tyrosine residues in marine organisms with concentration factors above 30 000 in some algae compared with sea water (13, 31). Our present findings suggest that iodine in drinking water may indicate marine organic matter at the source rock and thus humic substances of marine origin in drinking water.

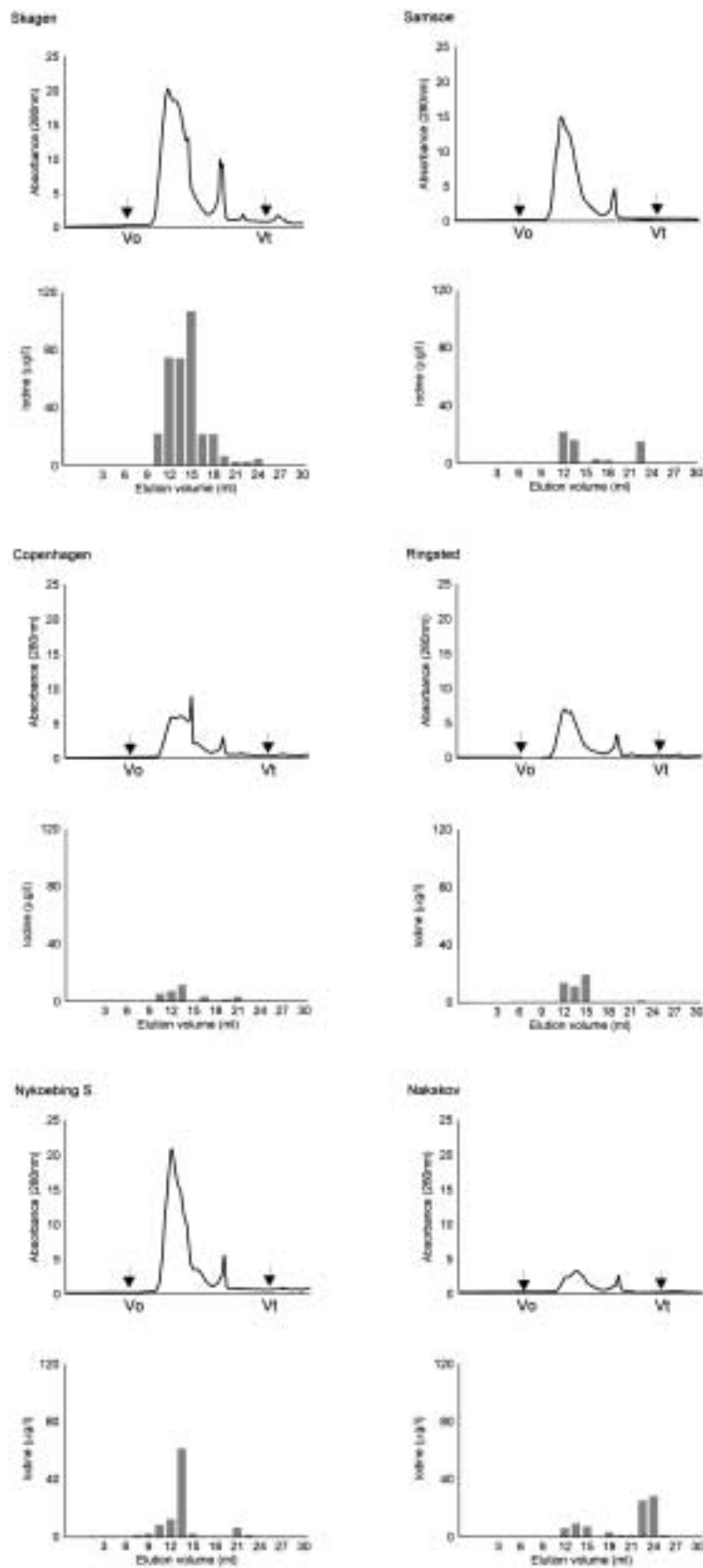


Figure 6 HPLC size exclusion (buffer: Tris 10 mM, pH 7.0) chromatograms of drinking water samples from six locations in Denmark. At all sites, a main peak in absorbance (280 nm) eluted at approximately 14 ml (K_{av} 0.34). In water from four sites, more than 90% of iodine eluted corresponding to this peak. In Nakskov 60% and in Samsø 25% of iodine eluted corresponding to low molecular weight substances.

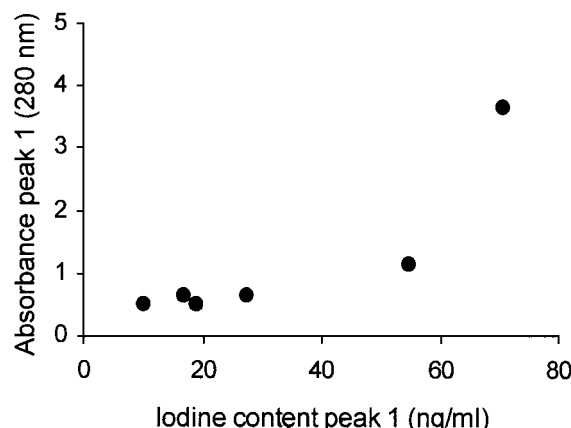


Figure 7 The relation between iodine content and the concentration of humic substances as evaluated from absorbance in HPLC size exclusion. Each dot represents one of the waterworks. Spearman's rho value was 0.85 with $P = 0.03$.

Our findings are in accordance with the geological evolution of the area. Present-day Denmark was covered with ice during four ice ages over the Quaternary period (1.6 million years until today). Ice depressed the Earth's crust by up to several hundred meters. When the ice melted the land rose again after a delay. During this delay, sea flooded the deglaciated terrain. This was followed by an uplift, exposing large areas of sea floor, and marine deposits have been found up to 60 m above sea level in present-day Denmark (32). Furthermore, reclamation and shore accumulation have added to marine sediments important for ground water resources in Denmark.

We previously found that iodine in drinking water correlated positively with urinary iodine excretion in Denmark (4). This suggests that the iodine in tap water is biologically available. This is in keeping with a study showing that humic acids can be absorbed by the gastrointestinal tract, although some may leave the body via the enterohepatic circulation (33). Further studies are needed to elucidate this in detail.

In general, iodine in drinking water relates inversely to goitre prevalence (7–11). However, both inconsistency and distinct exceptions were found (12, 34), and a possible explanation could be the inhibitory effect on thyroid function by some humic substances (21–23) coexisting with iodine as indicated.

In conclusion, iodine in drinking water from several locations in Denmark was part of humic substances. Our data indicate that iodine in drinking water suggests coexisting humic substances of marine origin.

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