



UNIVERSITÀ DI PARMA

ARCHIVIO DELLA RICERCA

University of Parma Research Repository

Analytical approaches for the characterization and quantification of nanoparticles in food and beverages

This is the peer reviewed version of the following article:

Original

Analytical approaches for the characterization and quantification of nanoparticles in food and beverages / Mattarozzi, Monica; Suman, Michele; Cascio, Claudia; Calestani, Davide; Weigel, Stefan; Undas, Anna; Peters, Ruud. - In: ANALYTICAL AND BIOANALYTICAL CHEMISTRY. - ISSN 1618-2642. - 409:(2017), pp. 63-80. [10.1007/s00216-016-9946-5]

Availability:

This version is available at: 11381/2821095 since: 2021-10-21T08:57:30Z

Publisher:

Springer Verlag

Published

DOI:10.1007/s00216-016-9946-5

Terms of use:

Anyone can freely access the full text of works made available as "Open Access". Works made available

Publisher copyright

note finali coverpage

(Article begins on next page)

Dear Author

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For **fax** submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style.
- Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL:

<http://dx.doi.org/10.1007/s00216-016-9946-5>

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information, go to:

<http://www.link.springer.com>.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us, if you would like to have these documents returned.

Metadata of the article that will be visualized in OnlineFirst

Graphical Abstract

1	Article Title	Analytical approaches for the characterization and quantification of nanoparticles in food and beverages	
2	Article Sub- Title		
3	Article Copyright - Year	Springer-Verlag Berlin Heidelberg 2016 (This will be the copyright line in the final PDF)	
4	Journal Name	Analytical and Bioanalytical Chemistry	
5		Family Name	Suman
6		Particle	
7		Given Name	Michele
8		Suffix	
9	Corresponding Author	Organization	Barilla G. R. F.Ili SpA, Advanced Laboratory Research
10		Division	
11		Address	via Mantova 166, Parma 43122
12		e-mail	Michele.Suman@barilla.com
13		Family Name	Mattarozzi
14		Particle	
15		Given Name	Monica
16		Suffix	
17	Author	Organization	Dipartimento di Chimica, Università degli Studi di Parma
18		Division	
19		Address	Parco Area delle Scienze 17/A, Parma 43124
20		e-mail	
21		Family Name	Cascio
22		Particle	
23		Given Name	Claudia
24		Suffix	
25	Author	Organization	RIKILT Wageningen UR - Institute of Food Safety
26		Division	
27		Address	P.O. Box 230, Wageningen 6700 AE
28		e-mail	
29		Family Name	Calestani
30		Particle	
31	Author	Given Name	Davide
32		Suffix	
33		Organization	IMEM-CNR, Parco Area delle Scienze 37/A
34		Division	

35		Address	Parma 43124
36		e-mail	
37		Family Name	Weigel
38		Particle	
39		Given Name	Stefan
40		Suffix	
41		Organization	RIKILT Wageningen UR - Institute of Food Safety
42		Division	
43	Author	Address	P.O. Box 230, Wageningen 6700 AE
44		Organization	BfR – Federal Institute for Risk Assessment
45		Division	
46		Address	Max-Dohm-Str. 8-10, Berlin 10589
47		Organization	BfR – Federal Institute for Risk Assessment
48		Division	
49		Address	Max-Dohm-Str. 8-10, Berlin 10589
50		e-mail	
51		Family Name	Undas
52		Particle	
53		Given Name	Anna
54		Suffix	
55	Author	Organization	RIKILT Wageningen UR - Institute of Food Safety
56		Division	
57		Address	P.O. Box 230, Wageningen 6700 AE
58		e-mail	
59		Family Name	Peters
60		Particle	
61		Given Name	Ruud
62		Suffix	
63	Author	Organization	RIKILT Wageningen UR - Institute of Food Safety
64		Division	
65		Address	P.O. Box 230, Wageningen 6700 AE
66		e-mail	
67		Received	17 June 2016
68	Schedule	Revised	29 August 2016
69		Accepted	14 September 2016
70	Abstract	Estimating consumer exposure to nanomaterials (NMs) in food products and predicting their toxicological properties are necessary steps in the assessment of the risks of this technology. To this end,	

analytical methods have to be available to detect, characterize and quantify NMs in food and materials related to food, e.g. food packaging and biological samples following metabolization of food. The challenge for the analytical sciences is that the characterization of NMs requires chemical as well as physical information. This article offers a comprehensive analysis of methods available for the detection and characterization of NMs in food and related products. Special attention was paid to the crucial role of sample preparation methods since these have been partially neglected in the scientific literature so far. The currently available instrumental methods are grouped as fractionation, counting and ensemble methods, and their advantages and limitations are discussed. We conclude that much progress has been made over the last 5 years but that many challenges still exist. Future perspectives and priority research needs are pointed out.

71	Keywords separated by ' - '	Nanoparticles - Nanomaterials - Emerging contaminants - Food - Analytical methods - Risk assessment
72	Foot note information	

Analytical approaches for the characterization and quantification of nanoparticles in food and beverages

Monica Mattarozzi¹ · Michele Suman² · Claudia Cascio³ · Davide Calestani⁴ · Stefan Weigel^{3,5} · Anna Undas³ · Ruud Peters³

Received: 17 June 2016 / Revised: 29 August 2016 / Accepted: 14 September 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Estimating consumer exposure to nanomaterials (NMs) in food products and predicting their toxicological properties are necessary steps in the assessment of the risks of this technology. To this end, analytical methods have to be available to detect, characterize and quantify NMs in food and materials related to food, e.g. food packaging and biological samples following metabolization of food. The challenge for the analytical sciences is that the characterization of NMs requires chemical as well as physical information. This article offers a comprehensive analysis of methods available for the detection and characterization of NMs in food and related products. Special attention was paid to the crucial role of sample preparation methods since these have been partially neglected in the scientific literature so far. The currently available instrumental methods are grouped as fractionation, counting and ensemble methods, and their advantages and limitations are discussed. We conclude that much progress has been made over the last 5 years but that many challenges still exist. Future perspectives and priority research needs are pointed out.

Keywords Nanoparticles · Nanomaterials · Emerging contaminants · Food · Analytical methods · Risk assessment

Introduction 34

Nanotechnology is a rapidly developing field and nanomaterials (NMs) are of significant economic interest with a global market value over 2 trillion euros in 2016 [1] and having an impact on many industries including the food industry [2]. In 2011, the European Commission (EC) released a specific recommendation on the definition of a nanomaterial: “a natural, incidental or manufactured material containing particles in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm” [3]. The EC recommendation intends to harmonize different European regulations, including REACH (Registration, Evaluation, Authorization and restriction of Chemicals) and CLP (Classification, Labelling and Packaging). In this context, analytical methods to detect, characterize and quantify NMs, as well as approaches for risk and exposure level assessment, will be required for the implementation and enforcement of such regulations [4–6]. A number of EU-funded projects are tackling this issue, including NanoDefine (<http://www.nanodefine.eu/>) which aims to establish analytical tools and guidance to support the implementation of the EC recommendation.

The current use of nanomaterials in the food sector can be related to three main areas: food structure, food additives and food packaging with various products for each category already available on the market. NM applications are found in the development of better tastes, enhanced flavour, texture and consistency of foodstuffs, in improved bioavailability of nutrients, in new food contact materials with particular barrier or

✉ Michele Suman
Michele.Suman@barilla.com

¹ Dipartimento di Chimica, Università degli Studi di Parma, Parco Area delle Scienze 17/A, 43124 Parma, Italy

² Barilla G. R. F.lli SpA, Advanced Laboratory Research, via Mantova 166, 43122 Parma, Italy

³ RIKILT Wageningen UR - Institute of Food Safety, P.O. Box 230, 6700 AE Wageningen, The Netherlands

⁴ IMEM-CNR, Parco Area delle Scienze 37/A, 43124 Parma, Italy

⁵ Present address: BfR – Federal Institute for Risk Assessment, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

64 mechanical properties, and in nano-sensor applications for
65 traceability and monitoring of food during transport and storage.
66 The current available information suggests that NMs used
67 in food and agriculture applications include both organic and
68 inorganic materials [7]. An application area of organic NMs is
69 the encapsulation of food additives. These so-called nutritional
70 delivery systems or nutraceuticals are generally micelles
71 composed of approved food-grade materials which are avail-
72 able as low-cost bulk ingredients [8]. The improved uptake
73 and bioavailability thanks to encapsulation of the active ingredi-
74 ents has opened up a large area of applications in food and
75 animal feed products that incorporate nano-sized vitamins,
76 nutraceuticals, antimicrobials, antioxidants, etc. [9–12].

77 Inorganic NMs known to be used in food, food additives,
78 food supplements and food packaging applications are silver,
79 iron, calcium and magnesium, selenium, silicates and titanium
80 dioxide [7]. Several food-grade nanoparticle products are al-
81 ready present on the market and thus presence of NMs in some
82 alimentary products can be considered as being likely [13].
83 For instance, nanomaterials such as synthetic amorphous silica
84 (SAS, or E551) are often added to foods that are in powder
85 form (e.g. salt, vegetable powder, egg powder, creamer, coffee
86 powder and so on) as an anticaking agent, thickener or carrier
87 of flavours. While E551 is one of the most important anticaking
88 agents, other manufactured anticaking agents include calcium
89 silicate (E552), sodium aluminosilicate (E554),
90 dicalcium phosphate (E341), sodium ferrocyanide (E535)
91 and microcrystalline cellulose (E460). Titanium dioxide
92 (TiO_2) in bulk form is approved as a food additive with num-
93 ber E171. It is used on a large scale as a whitener and as a
94 colorant to impart brightness to food products, especially con-
95 fectionary products. Part of the “food-grade” TiO_2 material
96 has been shown to be nano-sized [14, 15]. Nano-silver (Ag-
97 NM) is by volume not the most used material, but it is the
98 fastest growing NM application in food packaging owing to
99 its antimicrobial properties [16–19]. In the last few years there
100 has been increasing interest in the assessment of migration of
101 NMs from food contact materials (FCM) into food [20–22].
102 More recently, Ag-NMs have been studied as an alternative
103 for the antibiotics used in poultry production [23, 24]. Many
104 other metals in nano-sized particles are available as food or
105 health supplements. These include nano-selenium [25], nano-
106 calcium [26], nano-iron [27] and colloidal suspensions of met-
107 al particles, e.g. copper, gold, platinum, silver, molybdenum,
108 palladium, titanium and zinc [28]. As a result, it is likely that
109 consumers are exposed to such NMs on a daily basis [29, 30].

110 While the emerging nanotechnology holds many applica-
111 tions and benefits for the food sector, there are also concerns
112 about their safety. The main concerns stem from the lack of
113 knowledge regarding the interactions of NMs at the molecular
114 or physiological levels, and the fact that new NMs and appli-
115 cations thereof are constantly being produced [7, 31–33]. In
116 addition, the nanotechnology-derived foods are new to

117 consumers and it remains unclear how public perception, at-
118 titudes, choice and acceptance will impact the future of such
119 applications [34, 35]. To ensure sustainable development and
120 use of nanotechnology, especially in the food sector, requires
121 control and monitoring of NMs and risk assessments of their
122 application which in turn requires information about exposure
123 and toxicity. Even though a number of analytical methods for
124 the detection and characterization of NMs are available [4,
125 36], it is clear that it is necessary to improve the analytical
126 methods and strategies to enable risk assessments and imple-
127 ment future regulations [37]. Currently, risk assessments for
128 NMs are still very challenging, and complex issues and regu-
129 lations for NMs are constantly evolving [7]. Both issues im-
130 pose an urgent need to develop adequate analytical methodol-
131 ogies for detecting and characterizing NMs. This review aims
132 to summarize the current status of relevant analytical strategi-
133 es for detection, identification and characterization of NPs in
134 food products with particular attention to the crucial role of
135 sample preparation strategies for achieving reliable results.

136 Sample preparation

137 In the analysis of NMs in a food sample, it has to be taken into
138 account that these NMs are not common chemicals but highly
139 reactive physical objects of nano-sized dimensions and char-
140 acterized by a sometimes heterogeneous, evolving or vulner-
141 able nature (i.e. in the case of core shell nanoparticles, or in the
142 in vivo formation of a protein corona around inorganic parti-
143 cles). The physico-chemical properties of NMs may depend
144 on the surrounding matrix and can change over time in re-
145 sponse to slight perturbations of their environment. Thus,
146 the determination of NMs in food requires sample treatment
147 techniques that are able to extract or isolate NMs from com-
148 plex matrices which may contain many more particles of a
149 similar composition. At the same time, sample manipulation
150 should be minimized to guarantee analytical accuracy and
151 reduce the risks of artefacts [38]. For these reasons, the objec-
152 tive of sample preparation is to reduce sample complexity
153 with good recovery rates and reproducibility, while preserving
154 the original state and particle size distribution (PSD) of the
155 NMs in the initial food sample [6]. The time between sample
156 preparation and instrumental measurements and the extract
157 storage conditions are other important parameters to be inves-
158 tigated in order to prevent agglomeration, de-agglomeration,
159 dissolution and disruption phenomena, or undesired interac-
160 tions with other components in the matrix extract [39].
161 Another point of consideration is the minimum size of the
162 analytical sample that should be processed in order to be rep-
163 resentative for the whole sample. Linsinger et al. concluded
164 that the situation for NMs is comparable to that for molecules
165 and that usually sample sizes are large enough to contain
166 enough particles to allow approximation of the Poisson

167 distribution of particles by the normal distribution, which sig- 205
 168 nificantly reduces statistical complexity [40]. In addition, they 206
 169 mention that the minimum number of NMs in the subsample 207
 170 should be greater than 500 to limit the sampling error of the 208
 171 particle size distribution. Another approach to determine min- 209
 172 imum sample size is based on Gy's equation for particle sam- 210
 173 pling [41]. Although Gy's sampling theory is hard to digest, 211
 174 theoretical calculations show that a sample size of only 10 mg 212
 175 of a sample containing 100 nm Ag nanoparticles with a parti- 213
 176 cle mass concentration of 1 mg/kg is sufficient to achieve an 214
 177 analytical accuracy better than 10 %. In addition, the number 215
 178 of Ag nanoparticles in such a 10-mg sample is about 2×10^6 , 216
 179 far more than the minimum required number of 500 men- 217
 180 tioned by Linsinger [40]. Up to now, most of the published 218
 181 sample preparation procedures for nanoparticle analysis deal 219
 182 with aqueous environmental samples or stabilization of pure 220
 183 NM powders dispersed in pure water [42, 43]. However, in the 221
 184 last few years increasing efforts have been dedicated to more 222
 185 challenging solid environmental matrices, as well as to bio- 223
 186 logical and food matrices [4, 44]. 224

187 In the progressive preparation steps going from subsam- 225
 188 pling, particle extraction or matrix clean-up to final particle 226
 189 quantification, a number of quality check criteria should be in 227
 190 place to confirm (or at least to assess) particle size stability and 228
 191 recovery. In principle, stepwise sample preparation for NMs is 229
 192 not different from that required for classical analytes in food 230
 193 and beverages (Fig. 1) [45]. At first homogenization of the 231
 194 laboratory sample is required (step I), as is the actual extrac- 232
 195 tion or isolation of NMs from the matrix (step II). Comparable 233
 196 with classical contaminants or residues in food, a concentra- 234
 197 tion step may be required (step III). Finally, and this is differ- 235
 198 ent from other analytes, a stabilization of the NM suspension 236
 199 is often required (step IV). Step I generally consists of homog- 237
 200 enization of the laboratory sample, which may involve manual 238
 201 mixing or agitation and even heating or sonication. For step II, 239
 202 the isolation of NPs from complex food matrices, a simple 240
 203 water extraction combined with sonication has often proven 241
 204 inadequate, resulting in low recoveries and extracts containing

semi-solid matrix residues [39]. To overcome these limitations 205
 several methods are available and the choices often depend on 206
 the ruggedness of the NMs. 207

208 For inorganic NMs, sometimes called "hard" NMs, isola- 209
 tion is usually carried out by chemical or enzymatic digestion 210
 of the matrix. Traditional chemical digestion involves the use 211
 of strong mineral acids, often in combination with hydrogen 212
 peroxide and high temperatures [15, 45–47]. This, however, 213
 can cause the dissolution of NMs or reactions with dissolved 214
 sulfide and/or chloride species, thus losing information about 215
 their presence, size and concentration in the sample [48–50]. 216
 As a consequence, enzymatic and alkaline digestions have 217
 recently been proposed as valid alternatives for the analysis 218
 of reactive NMs in biological tissues and meat [24, 39, 219
 50–52]. Enzymatic digestion involves NM extraction and isola- 220
 tion by digestion of organic matrix constituents, such as 221
 proteins or carbohydrates. For this aim, broad spectrum en- 222
 zymes, as proteinase K and α -amylase have been used for the 223
 isolation of NMs in wheat, semolina, cookies, pasta [53] and 224
 chicken meat [24, 39]. For alkaline digestion, 225
 tetramethylammonium hydroxide (TMAH) is used and is able 226
 to efficiently digest soft tissue and selectively extract dis- 227
 solved metals without causing the dissolution of NMs to free 228
 ions [52]. The development of an efficient matrix digestion 229
 and NM isolation procedure requires the identification and 230
 optimization of critical parameters. Parameters such as tem- 231
 perature and time have been demonstrated to be important for 232
 digestion efficiency and reducing the risk of NM dissolution, 233
 precipitation or aggregation [24, 39, 51]. The addition of bo- 234
 vine serum albumin (BSA) prior to alkaline digestion is useful 235
 to prevent NM agglomeration due to the high ionic strength of 236
 TMAH solutions [51, 54]. In addition, the material and shape 237
 of the vials and tubes, the nature and concentration of surfac- 238
 tants, the methods of sample agitation, i.e. vortexing, mixing, 239
 stirring or sonication, can all affect the extraction recovery 240
 [48].

241 The literature describes only a few extraction procedures 241
 for the isolation of NMs from the matrix. Lopez-Lorente et al. 242

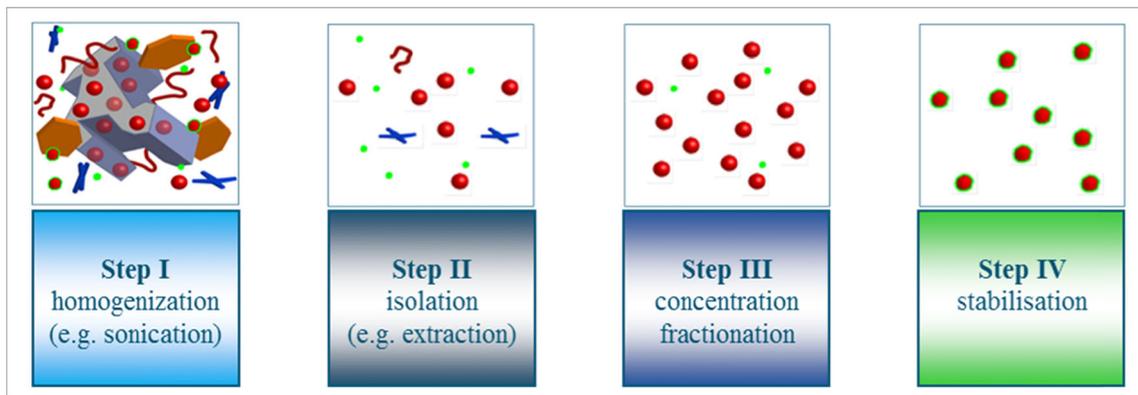


Fig. 1 Sample preparation for nanomaterials in food and consumer products. *Step I* sample homogenization (e.g. sonication). *Step II* extraction/isolation of nanomaterials. *Step III* nanomaterial concentration. *Step IV* nanomaterial stabilization

243 used a cationic surfactant in combination with an ionic liquid
244 in a micro liquid–liquid extraction to isolate gold nanoparti-
245 cles from water and liver [55]. In a more recent publication the
246 same author proposed to use nanomaterial-based sorbents for
247 the extraction of NMs of different nature [56]. The basic idea
248 is that nanomaterials or nanostructured matter can simulta-
249 neously act as an object (the analyte) in the sample and as a
250 (nano-)tool in different steps (sample preparation, separation
251 and detection) of the same analytical process. An example of
252 this is the extraction of AgNMs using a cationic surfactant in
253 combination with sulfonated nano-cellulose as an efficient
254 and environmental friendly dispersive micro solid-phase ex-
255 traction [57]. AgNMs extracted onto the nano-cellulose sor-
256 bent are desorbed into an aqueous solution containing thiotic
257 acid prior to capillary electrophoresis (CE) without the need
258 for any concentration or clean-up steps.

259 Besides matrix complexity and the nature of the nanopar-
260 ticle, the low concentration or heterogeneity of NMs in the
261 sample can be an additional issue to be addressed, requiring
262 prefractionation, purification and enrichment procedures such
263 as off-line settling, centrifugation or filtration [44].
264 Centrifugation and ultracentrifugation techniques depend on
265 the size, shape and density of the different sample compo-
266 nents, whereas in membrane filtration retention and elution
267 of an analyte depend on the size of membrane pores [58],
268 differentiating the size range of microfiltration (100 nm–
269 1 μm), ultrafiltration (1–100 nm) and nanofiltration (0.5–
270 1 nm) [4, 59]. Centrifugation permits one to reach enrichment
271 factors up to 10; however, it can also introduce the risk of
272 particle loss due to incomplete sedimentation, or even particle
273 alteration. Isolating nanoparticles by a filtration process is
274 called colloidal extraction. Filtration is the most common
275 prefractionation technique thanks to simplicity and low costs;
276 however, it is prone to artefacts caused by membrane clog-
277 ging, which decreases the effective pore size, by cake layer
278 formation on the membrane surface during filtration and by
279 membrane concentration polarization, thus modifying the size
280 distribution of the samples with respect to the centrifugation
281 [60–62]. For example, sequential filtrations of coffee creamer
282 extract through filters with decreasing pore sizes, i.e. 5, 0.45,
283 0.2 and 0.1 μm , have also been investigated to achieve selec-
284 tivity in the separation of the nano-silica fraction from the
285 matrix components [63]. However, this approach resulted in
286 losses of nano-silica during successive filtrations, probably
287 because of nanoparticle interactions with larger components
288 of the matrix and to poor quantitative performance of mem-
289 brane filtration [64]. Cross-flow (or tangential) filtration
290 (CFF) represents a valid alternative to dead-end filtration since
291 it gives the advantage of reduced clogging and concentration
292 polarization over the membrane, thanks to the tangential
293 movement of feed flow across membrane surface [44, 65,
294 66]. NM enrichment can also be achieved by cloud point
295 extraction, involving the addition of a surfactant to the sample

296 at a concentration that exceeds the critical micelle concentra-
297 tion. At a temperature higher than that for a specific cloud
298 point, the surfactant forms micelles in which non-polar sub-
299 stances are encapsulated. Since the density of the micelles is
300 higher than that of water, they settle after some time, a process
301 that is usually accelerated by centrifugation. Despite the high
302 enrichment factors (up to 100) that can be achieved by this
303 methodology, it is strongly influenced by matrix components
304 and particle surface properties. Up to now, this methodology
305 has been applied only for determination of silver NMs in
306 water, thus allowing their separation from ionic silver [67].
307 The final step of sample preparation often involves particle
308 stabilization to avoid dissolution or aggregation phenomena,
309 in order to minimize variability effects on the final measured
310 particle size distribution due to the sample preparation proce-
311 dure. If acid digestion has been used to remove the matrix, the
312 acid-digested sample has to be stabilized by adjustment of the
313 pH to a range compatible with the original particle suspension.
314 Particles may also be diluted and stabilized in a suitable dilu-
315 tion agent, for instance 0.01 mM sodium dodecyl sulfate
316 (SDS) and 0.025 % (v/v) FL-70TM as detergents, able to form
317 complexes and/or micelles, or 0.25 mM ammonium carbonate
318 as a buffer medium in order to adjust the ionic strength and pH
319 value [45]. As a result of all these aspects, any analytical
320 strategy is likely to be customized on the basis of the type
321 and nature of the NPs, the sample matrix, the instrumental
322 separation and detection techniques and the physico-
323 chemical properties to be assessed. Further research on the
324 optimization of sample preparation is currently being per-
325 formed within the NanoDefine project that will produce vali-
326 dated method and standard operating procedures (SOP) for
327 sample preparation of certain food matrices.

328 The compatibility of the prepared NM extracts and require-
329 ments of the instrumental analysis must be tested beforehand.
330 In some cases analyses of the extract with some separation
331 and/or detection technique can be unsuccessful or even im-
332 possible. As an example, the presence of digested or partially
333 degraded matrix can cause unresolved peaks due to non-ideal
334 elution or shifts of retention time in the asymmetric flow field-
335 flow fractionation (AF4) separation [39, 51]. In addition,
336 spike experiments of TiO_2 NMs on fish tissues demonstrated
337 that the extraction recovery depends on the type of tissue
338 investigated (gill, liver, muscle, spleen or intestine) with low-
339 est recovery for high-fat tissues such as liver [48]. For matrices
340 with a high fat content, a defatting step with an organic solvent
341 such as hexane could be included in the sample treatment
342 procedure [53, 63]. Residues of the matrix components can
343 be tolerated when they do not interfere with the instrumental
344 analysis and do not change the properties and aggregation
345 state of the particles.

346 In case of organic, so-called soft NMs, the sample prepa-
347 ration possibilities are limited since these types of nanoparti-
348 cles essentially consist of micelle-like structures that break up

349 easily [68]. Extraction procedures to isolate intact organic
 350 NMs from a sample matrix are lacking, probably because
 351 solvent extraction generally leads to a breakup of the NM
 352 structure [69]. For instance, to isolate Coatsome A liposomes
 353 from a beverage matrix, Helsper et al. used a combination of
 354 ultrafiltration and hydrodynamic chromatography (HDC),
 355 followed by mass spectrometry-based analysis for further
 356 identification and characterization [70].

357 **Analytical separation, detection and characterization**
 358 **of NMs**

359 Analytical methods for sizing and quantification of NMs in
 360 food can be divided in three groups: fractionation, counting
 361 and ensemble methods. In the fractionation group, the most
 362 applied technique is probably AF4 which has been combined
 363 with multi-angle light scattering (MALS) and inductively
 364 coupled plasma mass spectrometry (ICP-MS) for sizing and
 365 quantification of metal and metal oxide NMs (Fig. 2). Another
 366 separation technique is hydrodynamic chromatography
 367 (HDC) which has been combined with ultraviolet (UV) and
 368 ICP-MS detectors for the detection of organic NMs and metal
 369 and metal oxide NMs. The combination of a separation tech-
 370 nique with ICP-MS is a configuration often used, especially
 371 for metal and metal oxide NMs [71–74]. Another, less used
 372 fractionation method that will be discussed briefly is differ-
 373 ential centrifugal sedimentation (DCS). The applications of frac-
 374 tionation–detection combinations for NMs in food are de-
 375 scribed in more detail in the following sections.

376 The best example of the counting group is electron micros-
 377 copy (EM) which is recommended by the European Food
 378 Safety Agency (EFSA) for the size determination of NMs in
 379 food [75]. Normally, a prerequisite for counting methods is
 380 that the extracts need to be sufficiently clean to detect the NMs
 381 since matrix constituents that are still present in the extract will
 382 complicate the measurement or even make it impossible.
 383 Another counting technique is single particle ICP-MS

(spICP-MS) (Fig. 2). Since this technique is extremely sensi-
 384 tive for mass (typically nanograms per litre), extract dilution
 385 before spICP-MS analysis is often required, and this dilution
 386 will contribute to the clean-up of matrix constituents [76].
 387 Moreover since spICP-MS is element-selective, the presence
 388 of particulate matter of different chemistry will not hamper the
 389 detection, unless a clogging of the sample introduction system
 390 or the nebuliser occurs. Two counting techniques that will be
 391 discussed briefly are nanoparticle tracking analysis (NTA) and
 392 gas-phase electrophoretic mobility molecular analysis
 393 (GEMMA). The application of counting techniques for NMs
 394 in food is described in more detail in the following sections.
 395

The third group is ensemble techniques where large num-
 396 bers of NMs are measured simultaneously. Examples of that
 397 group are dynamic light scattering (DLS), particle-induced X-
 398 ray emission (PIXE), surface plasmon resonance (SPR) and
 399 coherent anti-Stokes Raman (CARS). DLS and CARS have
 400 not been used for NMs in food (DLS only in-line with a
 401 fractionation method, CARS solely for NMs in biological
 402 samples) and are therefore not discussed here. PIXE and
 403 SPR are briefly discussed in the following sections.
 404

Table 1 lists applications of FFF and HDC in combination
 405 with UV–Vis, MALS and ICP-MS, and applications of EM
 406 and spICP-MS for the detection and characterization of NMs
 407 in food. Table 2 gives an overview of the strong and weak
 408 points of the aforementioned techniques in the detection and
 409 characterization of NMs in food.
 410

411 **Fractionation methods**

412 **Field-flow fractionation**

In AF4 a cross-flow perpendicular to the carrier liquid is used
 413 to separate particles on the basis of their diffusion coefficient
 414 and hydrodynamic diameter [98–103]. Another field-flow
 415 fractionation technique that is used sometimes is sedimenta-
 416 tion FFF (Sd-FFF) which uses a centrifugal field for size
 417

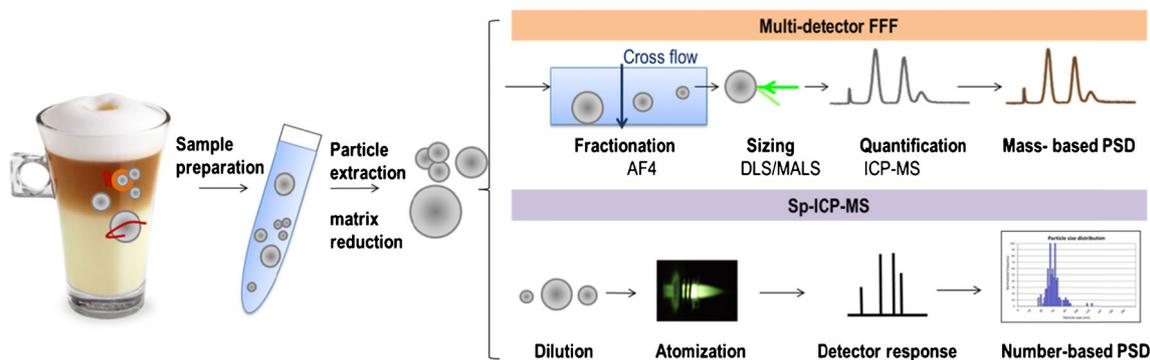


Fig. 2 Two possible analytical strategies for the sizing and quantification of NPs, indicated by grey spheres in the diagram, in food. Top AF4 with multiple detectors allows the determination of true size and a mass-based

particle size distribution (PSD). Bottom spICP-MS allows the determination of a spherical equivalent diameter of the particle and a number-based particle size distribution

418 separation of particles [73]. Note that FFF methods are per se
419 fractionation techniques able to achieve particle separation,
420 but not to independently determine particle size. Accurate
421 and independent size determination is only achieved by on-
422 line coupling to a DLS detector (hydrodynamic diameter)
423 [104], or better a multi-angle light scattering detector
424 (MALS) able to determine the radius of gyration [79, 98,
425 104]. Particle size can be estimated from AF4 theory by cal-
426 culating the hydrodynamic diameter based on retention time
427 and channel dimensions, or by calibrating the separation using
428 (certified) reference materials of known size [100]. Each ap-
429 proach has advantages and drawbacks. AF4 theory is based on
430 ideal running conditions and does not account for the particle
431 chemistry and surface charge properties that can cause large
432 shifts in elution time [39]. The use of size calibrants, mostly
433 polystyrene particles, is well established but also does not
434 account for particle chemistry and surface charge properties.
435 On-line coupling of a MALS detector allows for independent
436 determination of the radius of gyration but is relatively expen-
437 sive. Normally DLS is not suitable for samples of high com-
438 plexity, because the intensity of the scattered light in the nano-
439 range is proportional to the 6th power of the particle radius. As
440 a result, the presence of a few large particles will easily over-
441 shadow the presence of many small particles [105, 106]. On-
442 line coupling of FFF to a DLS detector is a convenient alter-
443 native, even if limited LOD (limit of detection) for size can
444 hamper the sizing of the smallest particles.

445 Carrier flow rates in AF4 are normally 0.5–1 mL/min mak-
446 ing AF4 compatible with MALS, UV-Vis, DLS and ICP-MS
447 detectors. UV-Vis can be helpful for detection/quantification
448 of organic NMs and plasmonic NMs, such as nano-gold and
449 nano-silver [100]. While inductively coupled plasma atomic
450 emission spectroscopy (ICP-AES) [104] and atomic absorp-
451 tion spectroscopy (AAS) [80] have been used, on-line ICP-
452 MS is the method of choice for element-specific detection and
453 quantification of metal-containing NMs [79, 100]. The LOD
454 for mass for the combination of AF4 with ICP-MS is in the
455 order of 10 µg/L for gold and silver. For silica and titania,
456 LODs are higher since the detection of Si and Ti is hampered
457 by the presence of polyatomic interferences. LODs in the
458 range of 0.16–0.3 mg/L for aqueous suspensions of silica
459 [79] and 0.5 mg/L for titania [14] are reported. The use of
460 collision cell technology and an MS/MS detector in ICP-MS
461 has resulted in improved LODs for silica nanoparticles [79].

462 The configuration AF4-MALS-ICP-MS can be used for
463 nano-separation, nanoparticle sizing and multi-elemental
464 quantification. Since ICP-MS detection determines mass,
465 the size distribution that is determined is actually a mass-
466 based size determination. This is a drawback since the EU
467 recommendation for a nanomaterial requires number-based
468 size distributions; therefore, a mathematical conversion is
469 required to translate mass-based into a number-based data
470 [14, 73]. Although this looks straightforward, the

471 uncertainty introduced by such a conversion results in a
472 limitation of the lower side of the particle size distribution
473 to 20 nm [14]. Other drawbacks of the AF4-MALS-ICP-
474 MS combination is that it is time consuming (AF4 runtimes
475 are typically 30–60 min), has poor dynamic size range
476 within a run at fixed conditions and is not able to distin-
477 guish constituent particles and aggregates/agglomerates. In
478 addition, optimization of the separation is time consuming
479 and it often has to be tuned for different NM/matrix com-
480 binations, which means that a sound knowledge of AF4,
481 the type of particle, its size and surface modification are
482 required. As a consequence, AF4 is more suitable as a
483 confirmatory technique and not as a screening technique.
484 The multidetector FFF approach has been applied for the
485 detection, sizing and quantification of NMs in food includ-
486 ing silica [45] in soup, titania in food, chewing gum and
487 toothpaste [14] and silver in chicken meat [39].
488 Sedimentation-FFF combined with off-line graphite fur-
489 nace atomic absorption spectrometry (GFAAS) has been
490 used for the characterization of silica particles
491 (Aerosil300, Aerosil380, Tixosil43 and Tixosil73) used
492 as food additives [80]. The effect of carrier pH, chemical
493 composition and conductivity played an important role in
494 the correct channel elution of silica. For these type of sam-
495 ples, preliminary preparation steps can significantly alter
496 the particle size distribution; moreover, elution conditions
497 including centrifugal field and carrier selection have to be
498 finely tuned to avoid fraction losses. Contado et al. [80]
499 achieved the best results with 0.1 % FL70 for Aerosil and
500 could confirm the presence of a fraction of primary parti-
501 cles of about 10 nm and aggregates/agglomerates in the
502 range 50–200 nm. The technique was also applied to a real
503 cappuccino sample.

504 Details of key studies are summarized in Table 1, highlight-
505 ing the particle–matrix addressed, type of detectors and elu-
506 ents used as well as key findings. This shows that great effort
507 is currently being put into the development and validation of
508 general methods for preparation and analysis of nanoparticles
509 in food, such as SiO₂ in tomato soup [45] or AgNPs in chicken
510 meat [77] as developed in the NanoLyse project. More work is
511 being carried out within the NanoDefine project on both pre-
512 paration method and analysis by multidetector FFF approaches:
513 further development of a generic sample preparation approach
514 to isolate nanomaterials from food and cosmetics using a ge-
515 neric multistep sample preparation procedure was successful-
516 ly demonstrated by Velimirovic et al. [107] for a powdered
517 tomato soup which contains the anticaking agent SiO₂ (E551)
518 and a sunscreen which contains TiO₂ as UV filter.

519 In general, the absence of official standardized protocols
520 for a more generic FFF separation, the lack of standards cer-
521 tified for size and mass or number concentration, and the
522 absence of fully inert membranes for AF4 currently represent
523 important technical bottlenecks for the widespread use of

Table 1 Summary of recent papers on sizing, detection and quantification of inorganic particle in food or food-relevant matrices by means of multidetector FFF, HDC detector, spICP-MS, EM and other techniques

	Particle	Matrix	Technique	Highlights	Ref.
t1.1	Asymmetric flow field-flow fractionation (AF4) or sedimentation FFF (Sd-FFF)	Chicken meat	AF4: $\text{NH}_4\text{CO}_3/\text{pH}$ 7: on-line MALS, US-Vis and ICP-MS	Detection and characterization of silver NPs in chicken meat by AF4 with detection by conventional or single particle ICP-MS; Non-ideal elution of NP in meat matrix on AF4	[39]
t1.2	Ag NPs 42 ± 10 nm	Chicken meat	AF4: $\text{NH}_4\text{CO}_3/\text{pH}$ 7: on-line US-Vis and ICP-MS	In-house validation of a method for determination of silver nanoparticles in chicken meat; findings demonstrate suitability of AF4-ICP-MS for quantitative determination of AgNPs	[77]
t1.3	Ag NPs 10, 40, 60 nm	Beer	AF4: 0.01 % SDS/pH 8: on-line US-Vis and ICP-MS	Characterization and quantification of AgNPs in nutraceuticals and beverages AF4-ICP-MS; it showed large dynamic range (10–1000 $\mu\text{g/L}$) and low detection limit, 28 ng/L	[78]
t1.4	SiO_2 (Aerodisp. W7520) wide size distribution, 136 nm (D_w , DLS)	Tomato soup	AF4: 0.025 % FL70/0.25 mM NaCl: on-line MALS and ICP-MS	Description of a generic sample preparation for inorganic engineered nanoparticles in a complex matrix	[45]
t1.5	Food grade SiO_2 and $\text{TiO}_2 < 20$ nm	Coffee creamer	A systematic approach to the determination of size and concentration of SiO_2 in a real food matrix AF4: demi water: on-line MALS and ICP-MS	Optimization of sample preparation including matrix-to-solvent ratio, defatting with organic solvents and sonication time	[63]
t1.6	SiO_2 20, 50, 80, 100, 120, 140, 160, 180 nm and ERM-FD100	Water	AF4: 0.02 % FL-70/0.2 mM NaCl/pH 8.5: on-line MALS, US-Vis and ICP-MS/MS	Quantitative characterization of silica nanoparticles; increased sensitivity due to MS/MS	[79]
t1.7	SiO_2 (Aerosil 300, -380, Tixosil 43, -73), wide size distribution	Pure E551 and cappuccino	Sd-FFF: 0.1 % FL70: off-line GFAAS	Size characterization by Sd-FFF of silica particles used as food additives; study of effects of sample preparation on the particle size distribution	[80]
t1.8	SiO_2 20, 40, 60, 80, 100 and 150 nm	Water	AF4: 0.25 mM NH_4CO_3 : on-line MALS, US-Vis and ICP-MS	Use of AF4-ICP-MS for the determination of size and concentration of SiO_2 -NPs with monomodal size distributions	[81]
t1.9	TiO_2 wide distribution, 60–300 nm	Pure E171 and 24 food products	AF4: 0.02 % FL70/0.02 % NaNO_3 : on-line ICP-MS	All TiO_2 in food products is particulate, and about 10 % is nano-sized	[82]
t1.10	TiO_2 wide distribution, prevalence of agglomerates/aggregates	Cosmetics/coffee cream and sugar glass	AF4: 0.2 % SDS/6 % methanol/pH 8.7: on-line US-Vis and ICP-MS	No NPs found in food, sonication time influenced PSD	[82]
t1.11	Hydrodynamic fractionation (HDC)	Milli-Q water	HDC (PL-PSDA type 1): 10 mM SDS: on-line spICP-MS	First on-line hyphenation of HDC and spICP-MS	[83]
t1.12	AuNPs 30, 60, 80 and 100 nm	Orange-flavoured beverage			[72]

Table 1 (continued)

Particle	Matrix	Technique	Highlights	Ref.
t1.17 Coatsome A, C, N -liposomes 153–205 nm		HDC (PL-PSDA type 1): 10 mM SDS; on-line UV, off-line MALDI-TOF	Characterization of three types of Coatsome liposome and validation of the HDC-UV-TOF-MS method	[84]
t1.18 Colloidal SiO ₂ Diameter 9–15 nm, length 40–100 nm	Milli-Q water	HDC (PL-PSDA type 1): 0.02 % NaN ₃ ; on-line MALS, QUELS, VISC and DRI	Complete characterization (size, shape, compactness and particle distribution) achieved in less than 20 min	[85]
t1.19 SiO ₂ (E551) 7 and 12 nm	Food products containing E551, E551 (Aerosil 200 F and 380 F)	HDC (PL-PSDA type 1): 10 mM SDS; On-line ICP-MS	Determination of nano-silica in food products with concentrations <0.1–1 mg/g and size ranges 50–200 nm	[86]
t1.20 SiO ₂ 10–200 nm E551/SAS and 32 nm SiO ₂	Food products with E551 digested via human in vitro digestion model	HDC (PL-PSDA type 1): 10 mM SDS; on-line ICP-MS	Changes in agglomeration state of nano-sized (5–200 nm) silica in different compartments mimicking the GI system	[87]
t1.21 Starch 57–165 nm	Waxy maize	HDC (25 × 1 cm, 5–15 µm Jordi-Gel GBR solid beads): 90 % DMSO/10 % MQ; on-line DALLS/MALLS	Ability to fractionate waxy maize amylopectins of MW > 100mln	[88]
t1.22 Single particle ICP-MS (spICP-MS)		spICP-MS; dwell time 3 ms	Validation of method for AgNPs in the range 5–25 mg/kg in chicken meat	[39]
t1.23 AgNPs (citrate-stabilized) 60 nm	Chicken meat	spICP-MS; dwell time 3 ms	More accurate sizing than AF4-ICP-MS, results compatible with TEM	[87]
t1.24 AgNPs (PVP-stabilized) (42 ± 10 nm)	Chicken meat	spICP-MS; dwell time 3 ms	AgNPs in the size range of 10 to 50 nm found. Results comparable with EM analysis	[88]
t1.25 AgNPs	Decoration pastry	spICP-MS; dwell time 50 µs	AgNPs accumulate in root tissue and tend to agglomerate, average particle size is 20 nm	[89]
t1.26 AgNPs 10 nm	Arabidopsis plants	spICP-MS; dwell time 3 ms	Determination of TiO ₂ in 24 food products showed that ~10 % of the particles have sizes <100 nm in a number-based particle size distribution	[89]
t1.27 TiO ₂ wide distribution 60–300 nm	Pure E171, 24 food products	spICP-MS; dwell time 3 ms	Measured in combination with ICP-OES, Zeta-sizer	[77]
t1.28 Electron microscopy		TEM: fixation; dehydration; resin infiltration and embedding; ultrathin section cutting; staining	Other techniques used are AF4-ICP-MS, ICP-MS	[87]
t1.29 Engineered Ag NPs 20 and 70 nm	Pear (skin and pulp)	SEM-EDS: fixation; dehydration; critical point drying; graphitization	Other technique used is spICP-MS. Size detection limit of EM better than spICP-MS	[78]
t1.30 Ag NPs 42 ± 10 nm	Chicken meat	TEM: enzymatic digestion (proteinase K); dilution	Concentration factor is interesting	[90]
t1.31 Ag NPs <20 nm	Silver pearls pastry decoration	TEM, STEM-EDS deposition of suspension on grid	Also measured with ICP-MS, AAS	
t1.32 Ag NPs 10, 40, 60 nm	Beer	TEM-EDS concentrated 10 times by evaporation; droplet on TEM grid; dried and covered with graphite coating		
t1.33 Ag NPs <100 nm	Apples, bread, carrot, soft cheese, meat, milk powder, orange juice, water	TEM-, SEM-EDS TEM-EDS (only orange juice): dilution in water		

t1.34 **Table 1** (continued)

Particle	Matrix	Technique	Highlights	Ref.
t1.34 Ag NPs 30–50 nm	Meat emulsion	ESEM-EDS: ashing for water containing samples TEM	Other technique used is EELS	[91]
t1.35 Inorganic NPs contaminants <100 nm	Bread and biscuits	Dilution, homogenization, centrifugation on grids ESEM-EDS	Oven drying used as a sample preparation technique	[92]
t1.36 Metallic NPs contaminants <100 nm	Wheat seeds, semolina, wheat flour, butter cookies, pasta	Oven drying SEM-EDS, ESEM-EDS treatment with TRIS buffer and heating; grinding (only for seeds); enzymatic digestion (α -amylase); defatting (only for butter cookies); filtration; filter graphitization	Also measured with ICP-MS	[53]
t1.37 NPs contaminants <10 nm	Drinking water	TEM ultracentrifugation directly on the grid	Other techniques: AFM, LIBD, STXM	[93]
t1.38 SiO ₂ NPs 80 nm	Tomato soup ASEM	deposition on the dish; dextran addition on the top of the sample TEM-EDS: ultracentrifugation; deposition on the copper grid	Other techniques: NTA	[94]
t1.39 Food grade SiO ₂ and TiO ₂ NPs <20 nm	Coffee creamer	TEM-EDS sample defatted with hexane; AF4 fraction centrifuged and filtered	Other techniques: AF4-ICP-MS, MALS	[63]
t1.40 Food grade-TiO ₂ NPs, 40–200 nm	Chewing gum	TEM-EDS	Other techniques: XRD, DLS	[95]
t1.41 ZnO and TiO ₂ NPs 20 nm and 50 nm respectively	Corn starch, yam starch, wheat flour	SEM-EDS Ashing at 750 °C for 16 h	Other techniques: ICP-OES	[96]
t1.42 TiO ₂ , CeO ₂ and ZnO NPs <100 nm	Fish	TEM, ESEM-EDS Digestion and filtration	Other techniques: ICP-MS, ICP-OES, DLS, CARS	[44]
t1.43 Other techniques	Orange juice and mussels	Capillary electrophoreses	Dispersive μ SPE technique for extraction	[57]
t1.44 AgNPs 10, 20, 60 nm	Water and fresh vegetables	Mercuric ion-based surface plasmon resonance (SPR) sensor	Can provide rapid and automated analysis dedicated to environmental and food safety monitoring	[97]
t1.45 AgNPs				

524 multidetector field flow fractionation as a routine analysis of
 525 NMs in food. Table 2 summarizes the pros and cons of AF4-
 526 ICP-MS in comparison to other reported methods.

527 **Hydrodynamic chromatography**

528 Particle size in HDC is correlated with the retention time,
 529 although interactions between the non-porous beads in the
 530 column and the analyte particles cannot be excluded. In the
 531 last few years HDC has become popular in environmental
 532 analysis to understand the behaviour, occurrence and fate of
 533 NMs [108–112]. The use of HDC for the analysis of complex
 534 samples was recently summarized by Laborda et al. [113].
 535 Although HDC can separate a broad particle size range and
 536 can be applied in a standard liquid chromatography configu-
 537 ration, HDC is not very popular in the analysis of food sam-
 538 ples. The main reason is that particle size separation in HDC is
 539 by far not as good as in AF4 [114]. Nevertheless, HDC has
 540 been used to study SiO₂ NMs in food products containing
 541 E551 [85], to investigate the fate of SiO₂ NMs after exposure
 542 to a human digestion model [30] and to separate liposome-
 543 based NMs [70]. The liposome-based NMs could not be sep-
 544 arated with AF4 since the shear forces in the AF4 channel
 545 broke up the micelle structure of the organic NM. Table 1
 546 summarizes the studies in which HDC coupled to different
 547 detectors was used in the analysis of food and environmental
 548 samples. Studies on both inorganic particles (SiO₂) and organ-
 549 ic particles (liposomes and starch) have been performed;
 550 moreover, the first on-line hyphenation between HDC and
 551 spICP-MS was recently demonstrated for AuNPs, and it has
 552 been included since its application has relevance for complex
 553 matrices including food.

554 **Differential centrifugal sedimentation**

555 DCS, also called centrifugal liquid sedimentation (CLS), can
 556 be used for particle size characterization of materials in the
 557 range of 5 to >1000 nm. The sample is injected in the centre of
 558 a rotating disk in which a gradient of sucrose is created and in
 559 which the particles are separated before reaching the edge of
 560 the disk where a detector is located. The actual particle size is
 561 calculated from the time needed to sediment the particle and
 562 the assumed material density. DCS can separate particles that
 563 differ in diameter by as little as 5 %, including separations in
 564 complex matrices such as plasma or cell culture media. The
 565 runtime of the analysis depends on the range of sizes being
 566 analysed and the density of the particles being measured [115,
 567 116]. For nanomaterials, analysis times are typically in the
 568 range of 15–30 min. The advantage of DCS is that in a rela-
 569 tively short time a high resolution is reached, and multimodal
 570 mixtures can be resolved [73]. DCS has been used for the
 571 characterization of silica nanoparticles suitable for food [117].

Counting methods

572

Electron microscopy

573

574 EM is the best technique to determine the shape, size and
 575 aggregation status of NPs. With a practical resolution of about
 576 10 nm in scanning electron microscopy (SEM) and 1 nm in
 577 transmission electron microscopy (TEM), the resolution is
 578 high enough to get detailed images of NMs in food [4, 6,
 579 118]. EM is recommended by the EFSA for the determination
 580 of particle size, shape and morphology of NMs in food, agro-
 581 chemicals and food packaging and for distinguishing them
 582 from other internal components such as liposomes, micelles
 583 or crystals [75]. EMs equipped with energy dispersive X-ray
 584 spectroscopy (EDS or EDX) become even more important
 585 tools for the determination of the elemental composition of
 586 the observed NMs. Dudkiewicz et al. presented an interesting
 587 overview of EM-based methods for the characterization of
 588 NMs in food, summarizing both sample preparation for EM
 589 and imaging approaches [118, 119]. In these reviews, the au-
 590 thors point out that the main challenge is the sample prepara-
 591 tion and that EM is best used as a complementary or confir-
 592 matory analysis to the analytical separation and detection
 593 techniques described in the text for these sections. In recent
 594 years, an increasing number of studies have been published
 595 concerning the determination of NMs in food matrices with
 596 EM and these are presented in Table 1. It shows that different
 597 approaches for sample preparation and different complemen-
 598 tary techniques and typical EM problems in the characteriza-
 599 tion of NMs have been investigated in these studies.

600 An important aspect of EM is the limited sample volume
 601 that can be analysed. This is a consequence of the fact that
 602 SEM and TEM at high magnification are more or less surface-
 603 related analytical tools. In SEM the penetration of the X-ray
 604 beam is a few micrometres while in TEM it is only a few tens
 605 of nanometres. As a result, only a limited number of NMs can
 606 be detected or visualized in such a small volume and the limit
 607 of detection is therefore high. Random sampling and investi-
 608 gation of several samples are necessary to obtain representa-
 609 tive results [119]. Automated image analysis software can
 610 improve the measurement statistics by analysing a large num-
 611 ber of areas on the sample [53]. However, with particle con-
 612 centrations at trace and ultratrace levels even this possibility
 613 runs into problems.

614 In conventional EM, samples are placed in a high vacuum
 615 and samples that are not electrically conductive must be coat-
 616 ed with a conductive layer to avoid charge accumulation.
 617 However, food samples may have a considerable amount of
 618 water which means that they have to be properly fixed and
 619 dried before the analysis. If the sample morphology is expect-
 620 ed to change as a result of the dehydration process, it is pos-
 621 sible to encapsulate hydrated samples in thin electron-
 622 transparent membranes, or to keep them in a solid form under

623 cryogenic conditions. Zhang et al. demonstrated this when
 624 they studied the contamination and penetration of AgNMs in
 625 pears [89]. For the EM analysis the pear samples were initially
 626 treated with a primary fixative solution and then subjected to
 627 dehydration, followed by critical point drying using liquid
 628 carbon dioxide. This allowed them to quantify the total con-
 629 tamination expressed as a number-based AgNM concentration
 630 and the penetration depth of AgNMs into the fruit. While 70-
 631 nm AgNMs were stopped on the skin of the fruit, 20-nm
 632 AgNMs penetrated the fruit and diffused into the pulp.

633 Nowadays a good alternative to the standard high vacuum
 634 EM is low vacuum often called environmental EM (ESEM) or
 635 atmospheric EM (ASEM) in which hydrated and even liquid
 636 samples can be observed at pressures up to 6000–7000 Pa and
 637 a relative humidity up to 100 % [108, 120]. Specific sample
 638 treatments that may affect NM size and distribution in the
 639 sample matrix can thus be avoided, although the resolution
 Q6 640 of ESEM and ETEM is generally not as good as that of the
 641 high vacuum equivalent. Using ESEM, Luo et al. [120]
 642 showed that mean sizes of SiO₂ ENP in tomato soup were
 643 larger when measured with ESEM compared to TEM and
 644 FEG-SEM. This provided useful additional knowledge on
 645 the aggregation state of NMs in the food matrix. Johnston
 646 et al. exposed fish to TiO₂, CeO₂ and ZnO NPs with sizes in
 647 the 20–100 nm range [47]. Using ESEM-EDS they not only
 648 observed that different kinds of NMs concentrated in different
 649 ratios in organs but also that active mucus production in re-
 650 sponse to irritation by the exposure to NMs produced large
 651 aggregates and precipitates which increased the average size
 652 of the NMs in water which in turn decreased the
 653 bioavailability.

654 In most of cases, NM analysis in food by EM is used as a
 655 qualitative, and not quantitative, analysis technique. This is
 656 especially the case when it is used independently from other
 657 measurements to confirm the presence of a certain NM or
 658 when complex matrices or low particle concentrations are in-
 659 volved. EM is then used to support other measurements, e.g.
 660 FFF-ICP-MS [63] or AF4-ICP-MS [77], and to visually con-
 661 firm the presence of NPs in the sample; however, there are
 662 exceptions. Beltrami et al., for instance, described the prepa-
 663 ration of thin SEM-ready layers of preconcentrated samples in
 664 order to perform multiple measurements of different areas
 665 [53]. In this way they collected statistically valid data on the
 666 concentration of metal NPs in raw materials and food prod-
 667 ucts, like common wheat, semolina, cookies and pasta.
 668 Similarly, Verleysen et al. described a validation method for
 669 the quantitative TEM measurement of Ag NPs in decoration
 670 pastry [87].

671 Single particle ICP-MS

672 Single particle ICP-MS (spICP-MS) has become popular for
 673 simultaneous sizing and quantifying of metal and metal oxide

NMs [14, 121, 122]. In spICP-MS the number of spikes ob- 674
 675 served in the time scan is directly proportional to the particle
 676 concentration in the sample, whereas the peak height is pro-
 677 portional to the particle's radius to the third power. This means
 678 that a number-based particle size distribution is determined
 679 which fits well with the EC definition of a nanomaterial [3].
 680 The particle size is calculated from the detected mass of the
 681 element that is measured assuming a certain composition and
 682 a spherical shape. However, without any a priori knowledge
 683 about a particle's composition and shape, no conclusions can
 684 be drawn about the true particle size. It is for this reason that
 685 spICP-MS is a screening method, albeit a very useful one.

686 An adequate time resolution and a low particle density in
 687 the sample are required to ensure that each signal originates
 688 from one particle only, hence the name *single particle* ICP-
 689 MS. While the runtime of a typical spICP-MS analysis is
 690 1 min, the time resolution used during the run is less than
 691 10 ms which can be handled by most standard ICP-MS sys-
 692 tems, and more recently less than 1 ms in specialized applica-
 693 tions on newer ICP-MS systems [123]. The short runtime
 694 makes spICP-MS analysis a much faster technique than any
 695 other for the detection of NMs. The limit of detection for mass
 696 is in the ng/L range which has the advantage that extracts can
 697 be diluted to minimize interferences from matrix constituents
 698 that may be present in the extract. The size detection limit of
 699 spICP-MS depends on a number of factors including the sen-
 700 sitivity of the mass detector, the mass fraction of the analyte in
 701 the particles, and the background noise in the time scan [124].
 702 For standard quadrupole ICP-MS systems the size-LOD is
 703 10–20 nm for gold and silver, 50 nm for titania and 200 nm
 704 for silica. Calculated size-LODs for 40 elements can be found
 705 in the literature [124]. An alternative to achieve lower size-
 706 LODs is the use of a high-resolution sector-field ICP-MS
 707 which is about 10 times more sensitive than a quadrupole
 708 resulting in two times lower LODs. Table 1 lists applications
 709 of spICP-MS described in the literature for the detection of
 710 NMs in food. Of special interest is the study of silver NMs in
 711 chicken meat since it describes the validation of the complete
 712 method according to EU regulation 2002/657/EC [125]. Two
 713 inter-laboratory studies have been organised to test the perfor-
 714 mance of spICP-MS for the determination of gold and silver
 715 NMs in aqueous extracts and in digested chicken liver [94,
 716 126]. A data evaluation tool has been developed for the cal-
 717 culation of particle size, particle size distribution and particle
 718 concentration from the raw spICP-MS data and is on-line
 719 available. Finally, an ISO standard is in preparation for the
 720 application of spICP-MS in aqueous extracts [127].

721 Recently spICP-MS has been used as a detector online with
 722 HDC as well as AF4 [83, 128]. Although data processing is
 723 still a challenge, the combination is an advantage because two
 724 independent particle sizes can be determined, one from the
 725 particle size separation (D_{HDC} or D_{AF4}), and a second from
 726 the spICP-MS analyses (D_{SP}). D_{HDC} and D_{AF4} are

t2.1 **Table 2** Synopsis of features and crucial points of AF4-ICP-MS, HDC-ICP-MS, EM-EDS and spICP-MS in the instrumental analysis of nanomaterials in food

t2.2 Performance characteristic	AF4-ICP-MS	HDC-ICP-MS	EM-EDS	sp-ICP-MS
t2.3 Determination of number-based particle size distribution as in EU recommendation for definition of nanomaterial	±	–	+	+
t2.4 Sensitivity ^a (size)	+	±	+	± (depends on element)
t2.5 Sensitivity ^b (mass)	±	±	±	+
t2.6 Discriminate between constituent particles and aggregates or agglomerates	–	–	+	–
t2.7 Discriminate between particles and ions	+	±	+	+
t2.8 Multi-elemental capability	+	+	+	± (in development)
t2.9 Capacity to size non-spherical particles	–	–	+	–
t2.10 Software for automated data processing	–	±	+	+
t2.11 Typical runtime per sample (min)	30–60	20	15	1
t2.12 Validated methods and standard operating procedures available ^c	±	–	+	±
t2.13 Specificity	+	+	– ^d	+
t2.14 Dynamic size range	±	+	–	+

– poor, ± moderate, + good

^a Sensitivity for size to be considered within the prospective of the EU definition of nanomaterials, wherein the lowest detectable size for nanomaterials has to be 1 nm. Both AF4-ICP-MS and EM-EDS can in certain conditions achieve such sensitivity; for spICP-MS the LOD for size depends on the constituent element of the particles and the presence of interferences, with typically 10–20 nm LODs for Au and Ag, 50 nm for TiO₂ and 200 nm for SiO₂

^b Sensitivity (for mass concentration) is considered in relation to the typical mass fraction (in weight) needed to clearly discriminate particles from the background; in the case of AF4-ICP-MS, HDC-ICP-MS, EM-EDS, reported mass LODs for mass are in the range of mg/L (for SiO₂ and TiO₂) and µg/L (for Au and Ag NPs); typical spICP-MS LODs for mass reported are in the ng/L range

^c Some in-house validated methods have been published; refer to Table 1 for details

^d If EDS is included in the EM analysis the – will change into +. Considering the limited resolution of EDS this is only expected for NPs with diameters >20 nm

727 hydrodynamic radii independent of the composition of the
 728 particle while D_{SP} is a spherical equivalent radius. If both radii
 729 are equal, the measured particle consists completely out of the
 730 measured element. If, however, $D_{SP} < D_{HDC}$ the particle con-
 731 sist only partly out of the measured element, as in the case of
 732 TiO₂, or the measured particle is actually an aggregate or
 733 agglomerate of the measured element. In addition, both com-
 734 binations can differentiate between nanoparticles and ions, an
 735 important topic for toxicologists.

736 **Nanoparticle tracking analysis**

737 NTA, or nanoparticle tracking analysis is a method for sizing
 738 particles in liquids by correlating the rate of the Brownian
 739 motion to particle size [129]. The technique calculates particle
 740 size on a particle-by particle basis and allows the determina-
 741 tion of a size distribution profile of particles with a diameter of
 742 approximately 30–1000 nm in liquid suspension. In relation to
 743 food, NTA has been used for the characterization of E551 in
 744 tomato soup [120] and the determination of Ag NMs in a
 745 chicken digest [94], and to study gold NMs in orange juice
 746 [130]. In all cases, particle size determination using NTA was
 747 reasonable (i.e. deviation less than 20 %), although the accu-
 748 racy was not as good as that from EM and spICP-MS in the
 749 same samples. As with dynamic light scattering, the presence

of large particles in the measurement cell easily results in 750
 overestimation from the size. The accuracy of particle concen- 751
 trations determined with NTA was poor compared to the other 752
 techniques and NTA gives no information of the chemical 753
 composition of the particle. 754

Gas-phase electrophoretic mobility analysis 755

GEMMA separates single charged analytes produced by a 756
 nano-electrospray process with subsequent drying of droplets 757
 and charge conditioning in a bipolar atmosphere by a ²¹⁰Po α- 758
 particle source. Size separation occurs in the gas-phase 759
 employing a constant, high-laminar flow of compressed air 760
 and a tuneable electric field. By variation of the electric field 761
 strength only nanoparticles of a corresponding electrophoretic 762
 mobility diameter (EMD) are able to pass the differential mo- 763
 bility analyser (DMA) unit of the instrument [131]. 764
 Depending on the DMA geometry, analytes in the size range 765
 of 10–500 nm can be analysed. Subsequent detection in a 766
 condensation particle counter (CPC) is number-, not mass- 767
 based allowing the analysis of nanoparticle samples without 768
 the bias of preferential detection of high molecular mass com- 769
 ponents [132]. Additionally, as detection occurs by scattering 770
 of a focused laser beam, even single particle detection is fea- 771
 sible. Correlation of obtained EMD values to molecular 772

773 weights (MWs) of respective standards, allows the mass de-
774 termination of analytes with unknown MW. Weiss et al. have
775 described the separation of protein-based, gelatine nanoparti-
776 cles [133].

777 Ensemble methods

778 Particle induced X-ray emission

779 PIXE is a technique that historically has been used to quantify
780 trace elements in materials, like traces of metal in archaeological
781 artefacts [134]. More recently it has been used for the detection
782 of nanomaterials [135]. PIXE is based on exciting electronic
783 levels of the atoms, by means of an ion beam, producing X-
784 rays that are characteristic and proportional to elements present
785 in the sample, thus allowing identification and quantification of
786 the elemental composition in a single measurement. The sensi-
787 tivity of PIXE is in the mg/L range and a typical runtime is 2–
788 5 min per sample. PIXE has been used to characterize nanopar-
789 ticles in rat lungs and faeces [136]. In another study Lozano et al.
790 used PIXE to quantify dispersions of silica and silver NMs in
791 coffee, milk and water. Since PIXE gives no information about
792 size, size analysis was performed using DCS [137].

793 Surface plasmon resonance

794 SPR has become a well-acknowledged screening tool in the
795 last decade that provides real-time and automated analysis
796 with relatively high capacity [138]. Incorporation of the bio-
797 logical recognition elements onto the sensor surface allows
798 detection of potentially biologically active compounds. For
799 instance, the detection of bioavailable heavy metals can be
800 achieved through the use of metal binding proteins such as
801 metallothioneins (MTs) [139]. Rebe-Raz et al. [97] showed
802 that AgNMs can be directly detected in their intact form using
803 hMT1A protein in combination with an SPR-based sensor.
804 The hMT1A sensor showed sensitivity in the parts per billion
805 range, displaying the highest sensitivity towards larger and
806 uncoated AgNMs. Potential applications of this sensor were
807 demonstrated by successfully detecting AgNMs in fresh veg-
808 etables and river water extracts within 10 min without the need
809 for complex sample preparation steps [97].

810 Conclusions and outlook

811 The application of nanotechnologies in the agri-food sector is
812 expected to increase. Current and future applications involve,
813 among others, inorganic bulk materials with size fractions
814 below 100 nm, nano-formulated minerals, and also organic
815 nano-carrier systems for vitamins, antioxidants and other food
816 supplements. A number of analytical methods have been

developed that can determine nanoparticles in a food matrix; 817
however, currently only electron microscopy is expected to be 818
suitable for classifying nanomaterials according to the EC 819
recommendation. Challenges that remain are (i) the complex- 820
ity of the matrix; (ii) the lack of certified reference materials 821
(both for size and mass); (iii) the scarcity of specific validated 822
methods for NPs in food and (iv) the development of new 823
analytical techniques and strategies. 824

The complexity of the food matrix, and with it the need for 825
sample preparation procedures, is a major issue. Until now 826
most research has been in the area of instrumental detection 827
and characterization of nanomaterials and not in the area of 828
sample preparation. Given the interactions between NMs and 829
many substances in food that can alter the physico-chemical 830
status of the NMs, more development in sample preparation 831
methods is needed. Presently each NM/matrix combination 832
requires its own method development and optimization; how- 833
ever, for the near future and the detection of “unknown” NMs, 834
generic sample preparation procedures are urgently needed. 835
New initiatives, like the use of “nano-tools” to extract NMs 836
are encouraging [56, 57]. In addition, the EFSA report about 837
the use of NMs in agriculture and food identified a trend 838
towards more organic NMs. At the moment methods for or- 839
ganic NMs are virtually absent [7]. New methods to detect, 840
characterize and quantify carbon-based NMs in food or to 841
characterize organic coatings of inorganic NMs are needed. 842

While aspects such as reproducibility and comparability of 843
NP measurements are important, presently there are only a 844
few validated methods [24]. A prerequisite for proper method 845
validation is the availability of reference materials. While cur- 846
rently only suspensions of the pure NMs are available as refer- 847
ence materials, a few studies have been undertaken to pro- 848
duce reference materials of Ag and SiO₂ NMs in food [140, 849
141], demonstrating that development and characterization of 850
a reference material in a food matrix is possible and that it is 851
feasible to assign reference values with acceptable uncer- 852
tainties. More reference materials are needed urgently (at this 853
stage certification is probably too difficult). 854

While there is a need for standardized methods (standard- 855
ized through ISO, CEN, etc.), these are not expected to be- 856
come widely available for NP in matrices as complex as food. 857
Therefore, a way forward would be the standardization of data 858
quality by agreeing on minimum performance requirements 859
for analytical methods and reference standards for method 860
validation. Approaches for the validation of analytical 861
methods for NMs in food have been proposed and applied 862
[24, 40]. To determine the data quality of (new) analytical 863
methods and harmonize their results, intercomparison studies 864
are needed. Presently two international intercomparison stud- 865
ies have been organized and executed, but more are needed 866
[94, 126]. Recognized reference laboratories could have a 867
prominent role in the organisation and implementation of the 868
suggested measures. 869

870 The currently most widespread techniques for the detection
 871 and characterization of NPs in food are EM, AF4-ICP-MS and
 872 spICP-MS. Among these spICP-MS appears to be closest to a
 873 routine application owing to its relative robustness, lower re-
 874 quirements for sample preparation, an increasing availability
 875 of evaluation software and an ISO technical specification de-
 876 scribing the spICP-MS procedure. AF4 still faces serious is-
 877 sues in reproducibility and requires trained and experienced
 878 operators. Improvements in membrane technology may dra-
 879 matically improve the situation. One of the former drawbacks
 880 of EM, high costs for operation and evaluation, are currently
 881 being overcome by the development of automated operation
 882 and image analysis techniques. In time this will render the
 883 technique accessible for a broader application range, including
 884 routine analysis. The enormous diversity of NMs with differ-
 885 ent sizes, shapes, compositions and coatings easily exceeds
 886 that of conventional chemicals. Therefore it is expected that
 887 analysis of NMs is not a question of a single analytical tech-
 888 nique but rather a combination of multiple procedures and
 889 instrumentation, and confirmation of the measurement result
 890 by a second technique which is based on a different physical
 Q7 891 principle is recommended.

892 **Compliance with ethical standards**

893 **Conflict of interest** The authors declare that they have no conflict of
 894 interest.

Q10/Q8/Q8 897 **References**

898 1. European Commission. Communication from the Commission to
 899 the European Parliament, the Council and the European Economic
 900 and Social Committee. Second regulatory review on
 901 nanomaterials; 2012.
 902 2. Cushen M, Kerry J, Morris M, Cruz-Romero M, Cummins E.
 903 Nanotechnologies in the food industry – recent developments,
 904 risks and regulation. *Trends Food Sci Technol.* 2012;24:30–46.
 905 3. European Commission. Commission Recommendation of 18
 906 October 2011 on the definition of nanomaterial. *Off J Eur*
 907 *Union.* 2011;L275/38-L275/40.
 908 4. Tiede K, Boxall AB, Tear SP, Lewis J, David H, Hasselov M.
 909 Detection and characterization of engineered nanoparticles in food
 910 and the environment. *Food Addit Contam Part A.* 2008;25:795–
 911 821.
 912 5. Stamm H, Gibson N, Anklam E. Detection of nanomaterials in
 913 food and consumer products: bridging the gap from legislation to
 914 enforcement. *Food Addit Contam Part A.* 2012;29:1175–82.
 915 6. Blasco C, Picó Y. Determining nanomaterials in food. *Trends Anal*
 916 *Chem.* 2011;30:84–99.
 917 7. Peters R, Brandhoff P, Weigel S, Marvin H, Bouwmeester H,
 918 Aschberger K, Rauscher H, Amenta V, Arena M, Botelho Moniz
 919 F, Gottardo S, Mech A. Inventory of nanotechnology applications
 920 in the agricultural, feed and food sector. EFSA supporting publi-
 921 cation 2014:EN-621. www.efsa.europa.eu/publications.

870 8. Deleers M, Pathak Y, Thassu D. Nanoparticulate drug delivery 922
 923 systems. New York: Informa Healthcare; 2007. ISBN 924
 9780849390739.
 925 9. Des Rieux A, Fievez V, Garinot M, Schneider YJ, Pr at V. 926
 927 Nanoparticles as potential oral delivery systems of proteins and 928
 929 vaccines: a mechanistic approach. *J Control Release.* 2006;116:1– 929
 930 27. 930
 931 10. Guo P, Martin CR, Zhao Y, Ge J, Zare RN. General method for 932
 933 producing organic nanoparticles using nanoporous membranes. 934
 935 *NANO Lett.* 2010;10:2202–6. 935
 936 11. Livney YD. Milk proteins as vehicles for bio-actives. *Curr* 937
 938 *Opinion Colloid Interface Sci.* 2010;15:73–81. 938
 939 12. Namazi H, Fathi F, Heydari A. Nanoparticles based on modified 939
 940 polysaccharides. In: *The delivery of nanoparticles.* InTech; 2012. 940
 941 ISBN 978-953-51-0615-9. Available from: [http://www.](http://www.intechopen.com/books/the-delivery-of-nanoparticles/nanoparticles-basedon-modified-polysaccharides) 941
 942 [intechopen.com/books/the-delivery-of-](http://www.intechopen.com/books/the-delivery-of-nanoparticles/nanoparticles-basedon-modified-polysaccharides) 942
 943 [nanoparticles/nanoparticles-basedon-modified-polysaccharides.](http://www.intechopen.com/books/the-delivery-of-nanoparticles/nanoparticles-basedon-modified-polysaccharides) 943
 944 13. Calzolari L, Gilliland D, Rossi F. Measuring nanoparticles size 944
 945 distribution in food and consumer products: a review. *Food* 945
 946 *Addit Contam Part A.* 2012;29:1183–93. 946
 947 14. Peters RJB, van Bommel G, Herrera-Rivera Z, Helsper HPGF, 947
 948 Marvin HJP, Weigel S, et al. Characterization of titanium dioxide 948
 949 NPs in food products: analytical methods to define NPs. *J Agric* 949
 950 *Food Chem.* 2014;62:6285–93. 950
 951 15. Weir A, Westerhoff P, Fabricius L, von Goetz N. Titanium dioxide 951
 952 NPs in food and personal care products. *Environ Sci Technol.* 952
 953 2012;46:2242–50. 953
 954 16. Duncan TV. Applications of nanotechnology in food packaging 954
 955 and food safety: barrier materials, antimicrobials and sensors. *J* 955
 956 *Colloid Interface Sci.* 2011;363:1–24. 956
 957 17. Hsueh Y-H, Lin K-S, Ke W-J, Hsieh C-T, Chiang C-L, Tzou D-Y, 957
 958 et al. The antimicrobial properties of silver nanoparticles in 958
 959 *Bacillus subtilis* are mediated by released Ag + ions. *PLoS One.* 959
 960 2015;10:e0144306. 960
 961 18. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation 961
 962 of antimicrobials. *Biotechnol Adv.* 2009;27:76–83. 962
 963 19. Siqueira MC, Coelho GF, de Moura MR, Bresolin JD, Hubinger 963
 964 SZ, Marconcini JM, et al. Evaluation of antimicrobial activity of 964
 965 silver nanoparticles for carboxymethylcellulose film applications 965
 966 in food packaging. *J Nanosci Nanotechnol.* 2014;14:5512–7. 966
 967 20. Echegoyen Y, Nerin C. Nanoparticle release from nano-silver 967
 968 antimicrobial food containers. *Food Chem Toxicol.* 2013;62:16–22. 968
 969 21. Bott J, Stormer A, Franz R. Migration of nanoparticles from plas- 969
 970 tic packaging materials containing carbon black into foodstuffs. 970
 971 *Food Addit Contam Part A.* 2014;31:1769–82. 971
 972 22. Mackevica A, Olsson ME, Hansen SF. Silver nanoparticle release 972
 973 from commercially available plastic food containers into food 973
 974 simulants. *J Nanopart Res.* 2016;18:article no. 5. 974
 975 23. Pineda L, Chwalibog A, Sawosz E, Lauridsen C, Engberg R, Elnif 975
 976 J, et al. Effect of silver nanoparticles on growth performance, 976
 977 metabolism and microbial profile of broiler chickens. *Arch* 977
 978 *Anim Nutr.* 2012;66:416–29. 978
 979 24. Peters RJB, Herrera Rivera Z, van Bommel G, Marvin HJP, 979
 980 Weigel S, Bouwmeester H. Development and validation of single 980
 981 particle ICP-MS for sizing and quantitative determination of nano- 981
 982 silver in chicken meat. *Anal Bioanal Chem.* 2014;406:3875–85. 982
 983 25. Xu J, Yang F, An X, Hu Q. Anticarcinogenic activity of selenium- 983
 984 enriched green tea extracts in vivo. *J Agric Food Chem.* 2007;55: 984
 985 5349–53. 985
 986 26. Hannig M, Hannig C. Nanomaterials in preventive dentistry. *Nat* 986
 987 *Nanotechnol.* 2010;5:565–9. 987
 988 27. Sekhon BS. Food nanotechnology – an overview. *Nanotechnol* 988
 989 *Sci Appl.* 2010;3:1–15. 989
 990 28. Purest Colloids. [http://www.purestcolloids.com/\(2013\)](http://www.purestcolloids.com/(2013)). Accessed 990
 991 1 Jan 2016. 991

- 987 29. Van der Zande M, Vandebriel RJ, Groot MJ, Kramer E, Herrera
988 Rivera ZE, Rasmussen K, et al. Sub-chronic toxicity study in rats
989 orally exposed to nanostructured silica. Part Fibre Toxicol.
990 2014;11:8.
- 991 30. Peters R, Kramer E, Oomen AG, Rivera ZE, Oegema G, Tromp
992 PC, et al. Presence of nano-sized silica during in vitro digestion of
993 foods containing silica as a food additive. ACS Nano. 2012;6:
994 2441–51.
- 995 31. House of Lord Science and Technology Committee. 1st Report of
996 Session 2009–10 Nanotechnologies and Food Volume I; 2010.
- 997 32. Smolkova B, El Yamani N, Collins AR, Gutleb AC, Dusinska M.
998 Nanoparticles in food. Epigenetic changes induced by
999 nanomaterials and possible impact on health. Food Chem
1000 Toxicol. 2015;77:64–73.
- 1001 33. FAO/WHO Expert Meeting on the Application of
1002 Nanotechnologies in the Food and Agriculture Sectors:
1003 Potential Food Safety Implications, Meeting Report 1-102,
1004 2009 ([http://www.fao.org/ag/agn/agns/nanotechnologies_en.
1005 asp](http://www.fao.org/ag/agn/agns/nanotechnologies_en.asp)).
- 1006 34. Chaudhry Q, Aitken R, Scotter M, Blackburn J, Ross B, Boxall A,
1007 et al. Applications and implications of nanotechnologies for the
1008 food sector. Food Addit Contam Part A. 2008;25:241–58.
- 1009 35. Friends of the Earth Australia. “Way too little”.
1010 [http://emergingtech.foe.org.au/wp-content/uploads/2014/05/
1011 /FOE_nanotech_food_report_low_res1.pdf](http://emergingtech.foe.org.au/wp-content/uploads/2014/05/FOE_nanotech_food_report_low_res1.pdf) (2014). Accessed 25
1012 Aug 2015.
- 1013 36. López-Serrano AL, Olivás RM, Landaluze JS, Nanoparticles CC.
1014 a global vision. characterization, separation and quantification
1015 methods. Potential environmental and health implications. Anal
1016 Methods. 2014;6:38–56.
- 1017 37. European Union. Scientific Committee on Emerging and Newly-
1018 Identified Health Risks (SCENIHR): Opinion on the appropriate-
1019 ness of the risk assessment methodology in accordance with the
1020 technical guidance documents for new and existing substances for
1021 assessing the risks of nanomaterials; 2007.
- 1022 38. Von der Kammer F, Lee Ferguson P, Holden PA, Masion A,
1023 Rogers KR, Klaine SJ, et al. Analysis of engineered nanomaterials
1024 in complex matrices (environment and biota): general consider-
1025 ations and conceptual case study. Environ Toxicol Chem.
1026 2012;31:32–49.
- 1027 39. Loeschner K, Navratilova J, Købler C, Mølhavé S, Wagner S, von
1028 der Kammer F, et al. Detection and characterization of silver NPs
1029 in chicken meat by asymmetric flow field flow fractionation with
1030 detection by conventional or single particle ICP-MS. Anal Bioanal
1031 Chem. 2013;405:8185–95.
- 1032 40. Linsinger TP, Chaudhry Q, Dehalu V, Delahaut P, Dudkiewicz A,
1033 Grombe R, et al. Validation of methods for the detection and
1034 quantification of engineered nanoparticles in food. Food Chem.
1035 2013;138:1959–66.
- 1036 41. Peters R, Helsper H, Weigel S. NanoLyse EU project. 2011.
1037 Nanoparticles in food: analytical methods for detection and char-
1038 acterisation. Deliverable D4.1a. Sampling of nanoparticles:
1039 Relation between sample size and sampling error. [http://www.
1040 nanolyse.eu/default.aspx](http://www.nanolyse.eu/default.aspx).
- 1041 42. Simonet BM, Valcárcel M. Monitoring NPs in the environment.
1042 Anal Bioanal Chem. 2009;393:17–21.
- 1043 43. Weinberg H, Galyean A, Leopold M. Evaluating engineered NPs
1044 in natural waters. Trends Anal Chem. 2011;30:72–83.
- 1045 44. Hassellöv M, Readman JW, Ranville JF, Tiede K. NPs analysis
1046 and characterization methodologies in environmental risk assess-
1047 ment of engineered NPs. Ecotoxicology. 2008;17:344–61.
- 1048 45. Wagner S, Legros S, Loeschner K, Liu J, Navratilova J, Grombe
1049 R, et al. First steps towards a generic sample preparation scheme
1050 for inorganic engineered nanoparticles in a complex matrix for
1051 detection, characterization, and quantification by asymmetric
flow-field flow fractionation coupled to multi-angle light scattering
and ICP-MS. J Anal At Spectrom. 2015;30:1286–96.
- 1052 46. López-Moreno ML, de la Rosa G, Hernández-Viezcás JA, Peralta-
1053 Videá JR, Gardea-Torresdey JL. X-ray absorption spectroscopy
1054 (XAS) corroboration of the uptake and storage of CeO₂ NPs and
1055 assessment of their differential toxicity in four edible plant species.
1056 J Agric Food Chem. 2010;58:3689–93.
- 1057 47. Johnston BD, Scown TM, Moger J, Cumberland SA, Baalousha
1058 M, Linge K, et al. Bioavailability of nanoscale metal oxides TiO₂,
1059 CeO₂, and ZnO to fish. Environ Sci Technol. 2010;44:1144–51.
- 1060 48. Shaw BJ, Ramsden CS, Turner A, Handy RD. A simplified meth-
1061 od for determining titanium from TiO₂ NPs in fish tissue with a
1062 concomitant multi-element analysis. Chemosphere. 2013;92:
1063 1136–44.
- 1064 49. Liu J, Pennell KG, Hurt RH. Kinetics and mechanisms of nano-
1065 silver oxysulfidation. Environ Sci Technol. 2011;45:7345–53.
- 1066 50. Gray EP, Coleman JG, Bednar AJ, Kennedy AJ, Ranville JF,
1067 Higgins CP. Extraction and analysis of silver and gold NPs from
1068 biological tissues using single particle inductively coupled plasma
1069 mass spectrometry. Environ Sci Technol. 2013;47:14315–23.
- 1070 51. Schmidt B, Loeschner K, Hadrup N, Mortensen A, Sloth JJ,
1071 Bender Koch C, et al. Quantitative characterization of gold NPs
1072 by field-flow fractionation coupled online with light scattering
1073 detection and inductively coupled plasma mass spectrometry.
1074 Anal Chem. 2011;83:2461–8.
- 1075 52. Arslan Z, Ates M, McDuffy W, Agachan MS, Farah IO, Yu WW,
1076 et al. Probing metabolic stability of CdSe NPs: alkaline extraction
1077 of free cadmium from liver and kidney samples of rats exposed to
1078 CdSe NPs. J Hazard Mater. 2011;192:192–9.
- 1079 53. Beltrami D, Calestani D, Maffini M, Suman M, Melegari B,
1080 Zappettini A, et al. Development of a combined SEM and ICP-
1081 MS approach for the qualitative and quantitative analyses of metal
1082 nano and microparticles in food products. Anal Bioanal Chem.
1083 2011;401:1401–9.
- 1084 54. Sager TM, Porter DW, Robinson VA, Lindsley WG, Schwegler-
1085 Berry DE, Castranova V. Improved method to disperse NPs for
1086 in vitro and in vivo investigation of toxicity. Nanotoxicology.
1087 2007;1:118–29.
- 1088 55. Lopez-Lorente AI, Simonet BM, Valcárcel M. Rapid analysis of
1089 gold nanoparticles in liver and river water samples. Analyst.
1090 2012;137:3528–34.
- 1091 56. Lopez-Lorente AI, Valcárcel M. The third way in analytical
1092 nanoscience and nanotechnology: involvement of nanotools and
1093 nanoanalytes in the same analytical process. Trends Anal Chem.
1094 2016;75:1–9.
- 1095 57. Ruiz-Palomero C, Soriano ML, Valcárcel M. Sulfonated
1096 nanocellulose for the efficient dispersive micro solid-phase ex-
1097 traction and determination of silver nanoparticles in food prod-
1098 ucts. J Chromatography A. 2016;1428:352–8.
- 1099 58. Kowalczyk B, Lagzi I, Grzybowski BA. Nanoseparations: strate-
1100 gies for size and/or shape-selective purification of NPs. Curr Opin
1101 Coll Interface Sci. 2011;16:135–48.
- 1102 59. Ferreira da Silva B, Pérez S, Gardinalli P, Singhal RK, Mozeto
1103 AA, Barceló D. Analytical chemistry of metallic NPs in natural
1104 environments. Trends Anal Chem. 2011;30:528–40.
- 1105 60. Morrison MA, Benoit G. Filtration artifacts caused by overloading
1106 membrane filters. Environ Sci Technol. 2001;35:3774–9.
- 1107 61. Chen JC, Li Q, Elimelech M. In situ monitoring techniques for
1108 concentration polarization and fouling phenomena in membrane
1109 filtration. Adv Colloid Interface Sci. 2004;107:83–108.
- 1110 62. Bolea E, Laborda F, Castillo JR. Metal associations to micropar-
1111 ticles, nanocolloids and macromolecules in compost leachates:
1112 size characterization by asymmetrical flow field-flow fractionation
1113 coupled to ICP-MS. Anal Chim Acta. 2010;661:206–14.
- 1114 63. Heroult J, Nischwitz V, Bartzak D, Goenaga-Infante H. The po-
1115 tential of asymmetric flow field-flow fractionation hyphenated to
1116 1117

1118 multiple detectors for the quantification and size estimation of
 1119 silica NPs in a food matrix. *Anal Bioanal Chem.* 2014;406:
 1120 3919–27.

1121 64. Von der Kammer F, Legros S, Larsen EH, Loeschner K, Hofmann
 1122 T. Separation and characterization of NPs in complex food and
 1123 environmental samples by field-flow fractionation. *Trends Anal*
 1124 *Chem.* 2011;30:425–36.

1125 65. Dalwadi G, Benson HA, Chen Y. Comparison of diafiltration and
 1126 tangential flow filtration for purification of nanoparticle suspen-
 1127 sions. *Pharm Res.* 2005;22:2152–62.

1128 66. Anders CB, Baker JD, Stahler AC, Williams AJ, Sisco JN, Trefry
 1129 JC, et al. Tangential flow ultrafiltration: a “green” method for the
 1130 size selection and concentration of colloidal silver nanoparticles. *J*
 1131 *Vis Exp.* 2012;68:e4167.

1132 67. Chao JB, Liu JF, Yu SJ, Feng YD, Tan ZQ, Liu R, et al. Speciation
 1133 analysis of silver nanoparticles and silver ions in antibacterial
 1134 products and environmental waters via cloud point extraction-
 1135 based separation. *Anal Chem.* 2011;83:6875–82.

1136 68. Luyckx DMAM, Peters RJB, van Ruth SM, Bouwmeester H. A
 1137 review of analytical methods for the identification and character-
 1138 ization of nano delivery systems in food. *J Agric Food Chem.*
 1139 2008;56:8231–47.

1140 69. Peters R, ten Dam G, Bouwmeester H, Helsper H, Allmaier
 1141 G, von der Kammer F, et al. Identification and characteriza-
 1142 tion of organic nanoparticles in food. *TRAC-Trend Anal*
 1143 *Chem.* 2011;30:100–12.

1144 70. Helsper JPF, Peters RJB, Brouwer L, Weigel S. Characterisation
 1145 and quantification of liposome-type nanoparticles in a beverage
 1146 matrix using hydrodynamic chromatography and MALDI-TOF
 1147 mass spectrometry. *Anal Bioanal Chem.* 2013;405:1181–9.

1148 71. Lespes G, Gigault J. Hyphenated analytical techniques for multi-
 1149 dimensional characterization of submicron particles: a review.
 1150 *Anal Chim Acta.* 2011;692:26–41.

1151 72. Sadik OA, Du N, Kariuki V, Okello V, Bushlyar V. Current and
 1152 emerging technologies for the characterization of nanomaterials.
 1153 *Sustainable Chem Eng.* 2014;2:1707–16.

1154 73. Cascio C, Gilliland D, Rossi F, Calzolari L, Contado C.
 1155 Experimental evaluation of key methods to detect, size and quan-
 1156 tify nanoparticulate silver. *Anal Chem.* 2014;86:12143–51.

1157 74. Cascio C, Geiss O, Franchini F, Ojea-Jimenez I, Rossi F, Gilliland
 1158 D, et al. Detection, quantification and derivation of number size
 1159 distribution of silver nanoparticles in antimicrobial consumer
 1160 products. *J Anal At Spectrom.* 2015;30:1255–65.

1161 75. EFSA Scientific Committee. Scientific opinion – Guidance on the
 1162 risk assessment of the application of nanoscience and nanotech-
 1163 nologies in the food and feed chain. *EFSA J.* 2011;9(5):2140.

1164 76. Peters R, Herrera-Rivera Z, Undas A, van der Lee M, Marvin H,
 1165 Bouwmeester H, et al. Single particle ICP-MS combined with a
 1166 data evaluation tool as a routine technique for the analysis of
 1167 nanoparticles in complex matrices. *J Anal At Spectrom.*
 1168 2015;30:1274–85.

1169 77. Loeschner K, Navratilova J, Grombe R, Linsinger TPJ, Købler C,
 1170 Mølhav K, et al. In-house validation of a method for determina-
 1171 tion of silver nanoparticles in chicken meat based on asymmetric
 1172 flow field-flow fractionation and inductively coupled plasma mass
 1173 spectrometric detection. *Food Chem.* 2015;181:78–84.

1174 78. Ramos K, Ramos L, Camara C, Gomez-Gomez MM.
 1175 Characterization and quantification of silver nanoparticles in
 1176 nutraceuticals and beverages by asymmetric flow field-flow frac-
 1177 tionation coupled with inductively coupled plasma mass spec-
 1178 trometry. *J Chromatogr A.* 2014;1371:227–36.

1179 79. Aureli F, D’Amato M, Raggi A, Cubadda F. Quantitative char-
 1180 acterization of silica nanoparticles by asymmetric flow field flow
 1181 fractionation coupled with online multiangle light scattering and
 1182 ICP-MS/MS detection. *J Anal At Spectrom.* 2015;30:1266–73.

80. Contado C, Ravani L, Passarella M. Size characterization by sed-
 1183 imentation field flow fractionation of silica particles used as food
 1184 additives. *Anal Chim Acta.* 2013;788:183–92. 1185

81. Barahona F, Geiss O, Urbán P, Ojea-Jimenez I, Gilliland D,
 1186 Barrero-Moreno J. Simultaneous determination of size and quan-
 1187 tification of silica nanoparticles by asymmetric flow field-flow
 1188 fractionation coupled to ICPMS using silica nanoparticles stan-
 1189 dards. *Anal Chem.* 2015;87:3039–47. 1190

82. Lopez-Heras I, Madrid Y, Cámara C. Prospects and difficulties in
 1191 TiO₂ nanoparticles in cosmetic and food products using asymmet-
 1192 rical flow field-flow fractionation hyphenated to inductively
 1193 coupled plasma mass spectrometry. *Talanta.* 2014;124:71–8. 1194

83. Pergantis SA, Jones-Lepp TL, Heithmar EM. Hydrodynamic
 1195 chromatography online with single particle-inductively coupled
 1196 plasma mass spectrometry for ultratrace detection of metal-
 1197 containing nanoparticles. *Anal Chem.* 2012;84:6454–62. 1198

84. Brewer AK, Striegel AM. Characterizing the size, shape and com-
 1199 pactness of a polydisperse prolate ellipsoidal particle via
 1200 quadrupole-detector hydrodynamic chromatography. *Analyst.*
 1201 2011;136:515–9. 1202

85. Dekkers S, Krystek P, Peters RJ, Lankveld DP, Bokkers BG, van
 1203 Hoeven-Arentzen PH, et al. Presence and risks of nanosilica in
 1204 food products. *Nanotoxicology.* 2011;5:393–405. 1205

86. Klavons JA, Dintzis FR, Millard MM. Hydrodynamic chromatog-
 1206 raphy of waxy maize starch. *Cereal Chem.* 1997;74:832–6. 1207

87. Verleysen E, Van Doren E, Waegeneers N, De Temmerman PJ,
 1208 Abi Daoud Francisco M, Mast J. TEM and SP-ICP-MS analysis of
 1209 the release of silver nanoparticles from decoration of pastry. *J*
 1210 *Agric Food Chem.* 2015;63:3570–8. 1211

88. Bao D, Oh ZG, Chen Z. Characterization of silver nanoparticles
 1212 internalized by arabidopsis plants using single particle ICP-MS
 1213 analysis. *Front Plant Sci.* 2016;7:32. 1214

89. Zhang Z, Kong F, Vardhanabhuti B, Mustapha A, Lin M.
 1215 Detection of engineered silver nanoparticle contamination in
 1216 pears. *J Agr Food Chem.* 2012;60:10762–7. 1217

90. Metak AM, Nabhani F, Connolly SN. Migration of engineered
 1218 nanoparticles from packaging into food products. *Food Sci*
 1219 *Technol.* 2015;64:781–7. 1220

91. Lari L, Dudkiewicz A. In: Nellist PD, editor. Electron microscopy
 1221 and analysis group conference 2013; sample preparation and
 1222 EFTEM of meta samples for nanoparticle analysis in food. IOP,
 1223 Bristol; 2014. 1224

92. Gatti AM, Tossini D, Gambarelli A, Montanari S, Capitani F.
 1225 Investigation of the presence of inorganic micron- and nanosized
 1226 contaminants in bread and biscuits by environmental scanning
 1227 electron microscopy. *Crit Rev Food.* 2009;49:275–82. 1228

93. Kaegi R, Wagner T, Hetzer B, Sinnet B, Tzetkov G, Böller M.
 1229 Size, number and chemical composition of nanosized particle in
 1230 drinking water determined by analytical microscopy and LIBD.
 1231 *Water Res.* 2008;42:2778–86. 1232

94. Peters RJB, Herrera-Rivera Z, Bouwmeester H, Weigel S, Marvin
 1233 HJP. Advanced analytical techniques for the measurement of
 1234 nanomaterials in complex samples: a comparison. *Qual Assur*
 1235 *Saf Crops Foods.* 2014;6:281–90. 1236

95. Periasamy VS, Athinarayanan J, Al-Hadi AM, Al Juhaimi F,
 1237 Mahmoud MH, Alshatwi AA. Identification of titanium dioxide
 1238 nanoparticles in food products: induce intracellular oxidative
 1239 stress mediated by TNF CYPIA genes in human lung fibroblast
 1240 cells. *Environ Toxicol Pharmacol.* 2015;39:176–86. 1241

96. Song X, Li R, Li H, Hu Z, Mustapha A, Lin M. Characterization
 1242 and quantification of zinc oxide and titanium oxide nanoparticles
 1243 in foods. *Food Bioprocess Technol.* 2014;7:456–62. 1244

97. Rebe-Raz S, Leontaridou M, Bremer MGE, Peters R, Weigel S.
 1245 Development of surface plasmon resonance-based sensor for de-
 1246 tection of silver nanoparticles in food and the environment. *Anal*
 1247 *Bioanal Chem.* 2012;403:2843–50. 1248

- 1249 98. von der Kammer F, Baborowski M, Friese K. Field-flow fractionation coupled to multi-angle laser light scattering detectors: applicability and analytical benefits for the analysis of environmental colloids. *Anal Chim Acta*. 2005;552:166–74. 1314
- 1250 99. Montoro Bustos AR, Ruiz Encinar J, Sanz-Medel A. Mass spectrometry for the characterization of nanoparticles. *Anal Bioanal Chem*. 2013;405:5637–43. 1315
- 1251 100. Dubascoux S, Le Hécho I, Potin Gautier M, Lespes G. On-line and off-line quantification of trace elements associated to colloids by As-FI-FFF and ICP-MS. *Talanta*. 2008;77:60–5. 1316
- 1252 101. Giddings JC. A new separation concept based on a coupling of concentration and flow nonuniformities. *Sep Sci*. 1966;1:123–5. 1317
- 1253 102. von der Kammer F. Characterization of the environmental colloids applying field-flow-fractionation - multi detection analysis with emphasis on light scattering techniques. Hamburg: Technischen Universität Hamburg; 2004. 1318
- 1254 103. Schimpf M, Caldwell K, Giddings JC. Field-flow fractionation handbook. New York: Wiley-Interscience; 2000. 1319
- 1255 104. Bednar AJ, Poda AR, Mitrano DM, Kennedy AJ, Gray EP, Ranville JF, et al. Comparison of on-line detectors for field flow fractionation analysis of nanomaterials. *Talanta*. 2013;104:140–8. 1320
- 1256 105. Calzolari L, Gilliland D, Pascual Garcia C, Rossi F. Separation and characterization of gold nanoparticle mixtures by flow-field-flow fractionation. *J Chromatogr A*. 2011;1218:4234–9. 1321
- 1257 106. Berne BJ, Pecora R. Dynamic light-scattering: with application to chemistry, biology and physics. New York: Dover; 2000. 1322
- 1258 107. Velimirovic M, Wagner S, von der Kammer F, Hofmann T. Applying a generic sample preparation approach to isolate nanomaterials from food and cosmetics. Conference Proceeding, SETAC Europe 25th Annual Meeting, Barcelona, Spain; 2015. 1323
- 1259 108. Tiede K, Tear SP, David H, Boxall ABA. Imaging of engineered nanoparticles and their aggregates under fully liquid conditions in environmental matrices. *Water Res*. 2009;43:3335–43. 1324
- 1260 109. Tiede K, Boxall ABA, Wang X, Gore D, Tiede D, Baxter M, et al. Application of hydrodynamic chromatography-ICP-MS to investigate the fate of silver nanoparticles in activated sludge. *J Anal At Spectrom*. 2010;25(7):1149–54. 1325
- 1261 110. Philippe A, Gangloff M, Rakcheeva D, Schaumann GE. Evaluation of hydrodynamic chromatography coupled with inductively coupled plasma mass spectrometry detector for analysis of colloids in environmental media - effects of colloid composition, coating and shape. *Anal Methods*. 2014;6:8722–8. 1326
- 1262 111. Philippe A, Schaumann GE. Evaluation of hydrodynamic chromatography coupled with UV-visible, fluorescence and inductively coupled plasma mass spectrometry detectors for sizing and quantifying colloids in environmental media. *PLoS One*. 2014;9:e90559. 1327
- 1263 112. Metreveli G, Philippe A, Schaumann GE. Disaggregation of silver nanoparticle homoaggregates in a river water matrix. *Sci Tot Environ*. 2015;535:35–44. 1328
- 1264 113. Laborda F, Bolea E, Cepriá G, Gómez MT, Jiménez MS, Pérez-Arantegui J, et al. Detection, characterization and quantification of inorganic engineered nanomaterials: A review of techniques and methodological approaches for the analysis of complex samples. *Anal Chim Acta*. 2016;904:10–32. 1329
- 1265 114. Gray EP, Bruton TA, Higgins CP, Halden RU, Westerhoff P, Ranville JF. Analysis of gold nanoparticle mixtures: a comparison of hydrodynamic chromatography (HDC) and asymmetrical flow field-flow fractionation (AF4) coupled to ICP-MS. *J Anal At Spectrom*. 2012;27:1532–9. 1330
- 1266 115. Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Bombelli FB, et al. Physical-chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. *J Am Chem Soc*. 2011;133:2525–34. 1331
- 1267 116. Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA. What the cell 'sees' in bionanoscience. *J Am Chem Soc*. 2010;132:5761–8. 1332
- 1268 117. Contado C, Mejia J, Garcia O, Piret JP, Dumortier E, Toussaint O, et al. Physicochemical and toxicological evaluation of silica nanoparticles suitable for food and consumer products collected by following the EC recommendation. *Anal Bioanal Chem*. 2016;408:271–86. 1333
- 1269 118. Dudkiewicz A, Tiede K, Loeschner K, Jensen LHS, Jensen E, Wierzbicki R, et al. Characterization of nanomaterials in food by electron microscopy. *TrAC-Trends Anal Chem*. 2011;30:28–43. 1334
- 1270 119. Dudkiewicz A, Boxall ABA, Chaudhry Q, Mølhav K, Tiede K, Hofmann P, et al. Uncertainties of size measurements in electron microscopy characterization of nanomaterials in foods. *Food Chem*. 2015;176:472–9. 1335
- 1271 120. Luo P, Morrison I, Dudkiewicz A, Tiede K, Boyes E, O'Toole P, et al. Visualization and characterization of engineered nanoparticles in complex environmental and food matrices using atmospheric scanning electron microscopy. *J Microsc*. 2013;250:32–41. 1336
- 1272 121. Pace HE, Rogers NJ, Jaromilek C, Coleman VA, Higgins CP, Ranville JF. Determining transport efficiency for the purpose of counting and sizing nanoparticles via single particle inductively coupled plasma-mass spectrometry. *Anal Chem*. 2011;83:9361–9. 1337
- 1273 122. Laborda F, Jiménez-Lamana J, Bolea E, Castillo JR. Selective identification, characterization and determination of dissolved silver(I) and silver nanoparticles based on single particle detection by inductively coupled plasma mass spectrometry. *J Anal At Spectrom*. 2011;26:1362–71. 1338
- 1274 123. Hineman A, Stephan C. Effect of dwell time on single particle inductively coupled plasma mass spectrometry data acquisition quality. *J Anal At Spectrom*. 2014;29:1252–7. 1339
- 1275 124. Lee S, Bi X, Reed RB, Ranville JF, Herckes P, Westerhoff P. Nanoparticle size detection limits by single particle ICP-MS for 40 elements. *Environ Sci Technol*. 2014;48:10291–300. 1340
- 1276 125. Commission of the European Communities. Commission Decision 2002/657/EC of 14 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Off J Eur Communities*. 2002;L221:8ff. 1341
- 1277 126. Linsinger TPJ, Peters R, Weigel S. International interlaboratory study for sizing and quantification of Ag nanoparticles in food simulants by single-particle ICPMS. *Anal Bioanal Chem*. 2014;406:3835–43. 1342
- 1278 127. International Standardization Organization. ISO/TS 19590: Nanotechnologies — size distribution and concentration of inorganic nanoparticles in aqueous media via single particle inductively coupled plasma mass spectrometry; 2015. 1343
- 1279 128. Huynh KA, Siska E, Heithmar E, Tadjiki S, Pergantis SA. Detection and quantification of silver nanoparticles at environmentally relevant concentrations using asymmetric flow field-flow fractionation online with single particle inductively coupled plasma mass spectrometry. *Anal Chem*. 2016;88:4909–16. 1344
- 1280 129. Vasco F, Hawe A, Jiskoot W. Critical evaluation of nanoparticle tracking analysis (NTA) by NanoSight for the measurement of nanoparticles and protein aggregates. *Pharm Res*. 2010;27:796–810. 1345
- 1281 130. Gallego-Urrea JA, Tuoriniemi J, Hassellöv M. Applications of particle-tracking analysis to the determination of size distributions and concentrations of nanoparticles in environmental, biological and food samples. *Trends Anal Chem*. 2011;30:473–83. 1346
- 1282 131. Allmaier G, Laschober C, Szymanski W. Nano ES GEMMA and PDMA, new tools for the analysis of nanobiotopes—protein complexes, lipoparticles, and viruses. *J Am Soc Mass Spectrom*. 2008;19:1062–8. 1347

- 1379 132. Allmaier G, Maißer A, Laschober C, Messner P, Szymanski WW. Parallel differential mobility analysis for electrostatic characterization and manipulation of nanoparticles and viruses. *TrAC-Trends Anal Chem.* 2011;30:123–32. 1380
1381
1382
1383 133. Weiss VU, Kerul L, Kallinger P, Szymanski WW, Marchetti-Deschmann M, Allmaier G. Liquid phase separation of proteins based on electrophoretic effects in an electrospray setup during sample introduction into a gas-phase electrophoretic mobility molecular analyzer (CE-GEMMA/CE-ES-DMA). *Anal Chim Acta.* 2014;841:91–8. 1384
1385
1386
1387
1388
1389 134. Demortier G. Application of nuclear microprobes to material of archaeological interest. *Nucl Instrum Methods Phys Res B.* 1988;30:434–43. 1390
1391
1392 135. Lozano O, Olivier T, Dogné JM, Lucas S. The use of PIXE for engineered nanomaterials quantification in complex matrices. *J Phys Conf Ser.* 2013;429:012010. 1393
1394
1395 136. Lozano O, Mejia J, Masereel B, Toussaint O, Lison D, Lucas S. Development of a PIXE analysis method for the determination of the biopersistence of SiC and TiC nanoparticles in rat lungs. *Nanotoxicology.* 2012;6:263–71. 1396
1397
1398
1418
137. Lozano O, Mejia J, Tabarrant T, Masereel B, Dogné JM, Toussaint O, et al. Quantification of nanoparticles in aqueous food matrices using particle-induced X-ray emission. *Anal Bioanal Chem.* 2012;403:2835–41. 1399
1400
1401
1402
138. Ricci F, Volpe G, Micheli L, Palleschi G. A review on novel developments and applications of immunosensors in food analysis. *Anal Chim Acta.* 2007;605:111–12924. 1403
1404
1405
139. Dabrio M, Rodríguez AR, Bordin G, Bebianno MJ, De Ley M, Sestáková I, et al. Recent developments in quantification methods for metallothionein. *J Inorg Biochem.* 2002;88:123–34. 1406
1407
1408
140. Grombe R, Charoud-Got J, Emteborg H, Linsinger TPJ, Seghers J, Wagner S, et al. Production of reference materials for the detection and size determination of silica nanoparticles in tomato soup. *Anal Bioanal Chem.* 2014;406:3895–907. 1409
1410
1411
1412
141. Grombe R, Allmaier G, Charoud-Got J, Dudkiwicz A, Emteborg H, Hofmann T, et al. Feasibility of the development of reference materials for the detection of Ag nanoparticles in food: neat dispersions and spiked chicken meat. *Accred Qual Assur.* 2015;20:3–16. 1413
1414
1415
1416
1417

UNCORRECTED PROOF

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

- Q1. Please check the suggested running page title if appropriate.
- Q2. Kindly provide the missing caption/legend of the online abstract figure
- Q3. The author Gy does not appear in list of authors for ref. 41. Please check that this is okay.
- Q4. What is “demi water” in Table 1 (ref. 64). Deionized?
- Q5. Please check the units in “100 mln” (see ref. 87, Table 1).
- Q6. Is “ETEM” correct? This abbreviation is not defined in the text. Similarly, please define the abbreviation FEG in “FEG-SEM” in the same paragraph.
- Q7. Please check that the last sentence of the “Conclusion and outlook” retains your intended meaning.
- Q8. References 15 and 47 based on original manuscript we received were identical. Hence, the latter was deleted and reference list and citations were adjusted. Please check if appropriate.
- Q9. References 30 and 116 based on original manuscript we received were identical. Hence, the latter was deleted and reference list and citations were adjusted. Please check if appropriate.
- Q10. References 109 and 123 based on original manuscript we received were identical. Hence, the latter was deleted and reference list and citations were adjusted. Please check if appropriate.
- Q11. Please provide the accessed date for all websites (e.g. ref. 28).
- Q12. If ref. 102 is a thesis, please change the reference accordingly.