

Human exposure assessment of silver and copper migrating from an antimicrobial nanocoated packaging material into an acidic food simulant



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ABSTRACT

To examine the human exposure to a novel silver and copper nanoparticle (AgNP and CuNP)/ polystyrene-polyethylene oxide block copolymer (PS-*b*-PEO) food packaging coating, the migration of Ag and Cu into 3% acetic acid (3% HAC) food simulant was assessed at 60 °C for 10 days. Significantly lower migration was observed for Ag (0.46 mg/kg food) compared to Cu (0.82 mg/kg food) measured by inductively coupled plasma – atomic emission spectrometry (ICP-AES). In addition, no distinct population of AgNPs or CuNPs were observed in 3% HAC by nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). The predicted human exposure to Ag and Cu was used to calculate a margin of exposure (MOE) for ionic species of Ag and Cu, which indicated the safe use of the food packaging in a hypothetical scenario (e.g. as fruit juice packaging). While migration exceeded regulatory limits, the calculated MOE suggests current migration limits may be conservative for specific nanopackaging applications.

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1. Introduction

Nanotechnology, involving the manipulation of matter in the size range 1–100 nm, is being hailed as a precursor for the next “industrial revolution” (Karkare, 2008). The unique properties of engineered nanoparticles (NPs) has driven their inclusion in a number of products worldwide (Maynard and Michelson, 2014). In particular, the use of NPs in food packaging to provide active and intelligent properties has seen growing interest in recent years due to potential improvements in food safety at every stage from supplier to consumer (Hannon et al., 2015a). Metal NPs, such as silver NPs (AgNPs), can be identified as a popular NP due its antimicrobial ability when incorporated in food packaging. Significant antimicrobial activity of AgNPs against gram positive and gram negative bacteria has been observed on coated packaging materials

(Bondarenko et al., 2013; Azlin-Hasim et al., 2015). Another metal that has been considered as an alternative to Ag at the nanoscale for its antimicrobial activity is CuNPs (Bergin and Witzmann, 2013). Antimicrobial activity of CuNPs has been proven effective against gram positive and gram negative bacteria and yeast (Usman et al., 2013). However, its benefits such as availability and antimicrobial activity are stifled by CuNPs ability to agglomerate and rapidly oxidise, reducing their usability. Coatings incorporating block copolymer self-assembled nanopatterns have the ability to produce uniformly distributed metal nanodots, counteracting any potential reduction of novel properties caused by agglomeration (Ghoshal et al., 2012, 2013; Azlin-Hasim et al., 2015).

Despite the benefits associated with the antimicrobial activity of metal NPs, such as improved food safety and shelf life, there are concerns related to the migration of NPs from nanocoated food packaging into food and their potential human toxicity once consumed. These concerns are not aided by the uncertainty which surrounds NP mechanisms of toxicity. For Ag, it is widely recognised that the most toxic state is ionic silver (Ag⁺) (Xiu et al., 2012),

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where AgNPs have the ability to penetrate natural barriers, accumulate and provide a gradual intracellular release of Ag⁺ (Hadrup and Lam, 2014). As a result, AgNP toxicity is highly dependent on its ability to permeate into cells, which differs greatly from Ag⁺ which is unable to permeate cell membranes and so relies heavily on extracellular toxicity (Caballero-Díaz et al., 2013). In the literature, there are a number of studies reporting the toxicity of NPs against human gastrointestinal cells *In vitro* (Böhmert et al., 2014) and against mammals *In vivo* (Kim et al., 2008; Shahare and Yashpal, 2013). In contrast, a study by Munger et al. (2014) which investigated the *In vivo* human toxicity of orally dosed commercially available AgNPs observed no clinically important changes in both groups after exposure to 10 mg/l of 5–10 nm AgNPs (Group No. 1) and to 32 mg/l of 25–40 nm AgNPs after repeated dosing for 3, 7 and 14 day; and 14 day time periods, respectively. The average daily ingestion of elemental Ag was estimated as 100 µg/day and 480 µg/day for the 10 mg/l and 32 mg/l colloid, respectively. From a food safety perspective there needs to be an assessment of NP migration from NP packaging and determination of corresponding potential human exposure. A number of studies are present in the literature which have assessed the migration potential of NPs from food contact materials (FCMs) (Hannon et al., 2015a,b). Only a select number of studies have assessed the migration of NPs from NP coatings on FCMs (Hannon et al., 2015a,b; Nobile et al., 2004; Smirnova et al., 2012). This shortfall may be due to the lack of suitable methods to characterise and quantify NPs in the food packaging, foodstuff and food simulants.

An imperative element of nanomaterial risk assessment is the exposure assessment, which can be based on migration studies. In any human exposure assessment the level of exposure is determined based on the three factors which include; NP migration, consumption of the foodstuff containing the migrant NPs and the body weight of the individual consuming the foodstuff. There has been a limited number of studies that have assessed the human exposure to nanomaterial present in FCMs. Existing studies have assessed human exposure to unintentionally ingested AgNPs in children's sippy cups (Bachler et al., 2013; Tulve et al., 2015), AgNPs in food storage containers and storage bags (von Goetz et al., 2013) and experimental AgNP FCMs (Cushen et al., 2013, 2014; Smirnova et al., 2012). More human exposure studies are necessary to form a better understanding of the human toxicity and exposure potential from NPs in consumer products.

The objective of this study was to investigate the migration potential of antimicrobial Ag or Cu nanodot coated surfaces developed using the self-assembly of a polystyrene-*b*-polyethylene oxide (PS-*b*-PEO) block copolymer into an acidic food simulant and predict the hypothetical human exposure from consumption of a food type which could potentially use this type of packaging (e.g. fruit juice).

2. Materials and methods

2.1. Nanocomposite manufacture

Block copolymer (BCP) PS-*b*-PEO ($M_n = 32\text{--}11$ kg/mol) was purchased from Polymer Source Inc., Canada; AgNO₃ (99%), acetone (99%), toluene (99%) and anhydrous ethanol ($\geq 99.8\%$) were obtained from Sigma-Aldrich, Ireland; and CuSO₄·5 H₂O ($\geq 98.5\%$) from Fisher Scientific, Ireland.

The metal nanodots were fabricated as described in Ghoshal et al. (2013) and Azlin-Hasim et al. (2015). Microscope glass slides (2.5 cm × 2.5 cm) were cleaned with ethanol and then acetone for 30 min in an ultrasonicator. Then, the substrate were spin coated at 3000 rpm for 30 s with BCP PS-*b*-PEO (1 wt % in toluene). Following the spin coating, the coated BCP were microphase separated using a

solvent-annealing technique in a closed system vessel at 50 °C for 1 h and then etched with ethanol for 15 min in an ultrasonicator to remove minor components of PEO and form nanocylinders. The substrate was then dried under a stream of N₂ gas. Subsequently, ethanolic solutions of metal precursor (i.e. AgNO₃ (precursor of Ag) and CuSO₄·5 H₂O (precursor of Cu)) (0.5% w/w) were spin coated at 3000 rpm for 30 s to fill in the formed nanocylinders with the metal precursor. The coated surface was then exposed to UV/Ozone for 3 h to remove any presence of organic materials and residues of solvent. The UV/Ozone treatment also oxidised the Ag⁺/Cu²⁺ to Ag/Cu nanodots.

2.2. Determination of metal NP loading in coating

To determine the initial concentration of AgNPs/CuNPs in the PS-*b*-PEO surface coating, samples were placed in individual round bottom flasks and 6 ml of nitric acid (HNO₃) (69% HNO₃, VWR International, Dublin, Ireland) was added. A water cooled reflux and watch glass were placed over the round bottom flask and digestion was carried out using a heating mantle at 95 ± 5 °C. After 30 min, 2 ml of hydrochloric acid (HCl) (37% HCl, Sigma-Aldrich, Arklow, Ireland) was added and digestion was continued for 2 h. Following digestion the solution was allowed to cool before the reflux and watch glass were removed. The glass slide used as a coating substrate, watch glass and reflux were all rinsed with Milli-Q water (MQW) (MQW 18.2 MΩcm, PURELAB Option-Q, Elga, UK) into the round bottom flask to remove any residual Ag or Cu which may have been transferred to them during digestion. In a similar process, the digestate was transferred to a 100 ml volumetric flask and the round bottom flask was rinsed thoroughly to remove residual Ag and Cu. The digestate was then made up to the 100 ml volume and a 5 ml aliquot was isolated for ICP-AES analysis.

2.3. Migration study

To quantify the migration of Ag and Cu from the nanocoated glass slides, migration studies were carried out according to European Commission Regulation No. 10/2011 (European Commission (EU) 2011). Each nanocoated sample was placed, coated side up, in a polyethylene container and 6.25 ml of 3% v/v acetic acid (HAc) prepared volumetrically in distilled water (>99% HAc, Sigma-Aldrich) was added. Samples were then incubated in an oven (Plus II Oven, Gallenkamp, Loughborough, UK) for 10 days at 60 °C. After the 10 day incubation period, samples were removed from the sample pots and 5 ml of the simulant was transferred to a 10 ml pyrex tube followed by 50 µl of HCl (37% HCl, Sigma-Aldrich) and 100 µl of HNO₃ (69% HNO₃, VWR International). Pre-digestion was carried out on each of the samples for 2.5 h at 95 ± 5 °C using a test tube heater (Palintest Digital Tubetest Heater, Gateshead, UK) prior to ICP-AES analysis.

2.4. Inductively coupled plasma – atomic emission spectrometry

The total Ag/Cu migration and initial concentration of Ag/Cu in the coating was determined using an ICP-AES (Vista Pro RL, CCD simultaneous ICP-AES, Varian, Victoria, Australia). Before analysis, the ICP-AES was allowed to equilibrate for 50 min. For each 10 samples, the ICP-AES was calibrated using four standards (0.01, 0.1, 1 and 5 mg/l) prepared volumetrically by serial dilution of a stock standard solution (1000 mg/l Ag⁺ in HNO₃, 1000 mg/l Ga⁺ in HNO₃ and 5 mg/l Cu in multi-element standard, Elementec, Kildare, Ireland). To combat matrix effects yttrium (1000 mg/l Y in HNO₃, Elementec) was added as an internal standard to all samples, blanks and calibration standards. To remove any potential sources of contamination in the study, all of the apparatus and sample pots

which contacted the food simulant was soaked in a 5% HNO₃ prepared in distilled water for 24 h, followed by rinsing with drop-wise concentrated HNO₃ and copious amounts of distilled water.

2.5. Nanoparticle tracking analysis (NTA)

NTA was carried out using a NanoSight NS300 equipped with a syringe pump. A volume of 1 ml of the respective sample was aspirated into a 1 ml syringe and air bubbles were removed before being loaded onto the syringe pump. Approximately 500 µl was pumped through the system to allow sample to reach the flow cell. Once in the viewing window, the NPs were manually brought into focus before being captured using the settings as outlined in Table 1. Before and after each run, the NTA was cleaned using 1 ml of HPLC grade 10% EtOH and 2 ml of HPLC grade H₂O. Once the videos were captured, each video was processed in the NTA 3.1 program. Post-processing of the NTA data was carried out in MS Excel 2013.

2.6. Scanning electron microscopy

The presence of NPs in both the Ag and Cu nanodot coating was determined by scanning electron microscopy (Hitachi S-4300 field emission SEM, Hitachi High Technologies America, Inc., USA). The NP coated samples were placed, nanodot coated substrate side up, on carbon tape and gold coated (Agar sputter coater with gold target, Agar Scientific, Essex, UK) before being imaged. SEM images were analysed using the freeware programme Fiji (Schindelin et al., 2012). In Fiji, differences in contrast and noisy pixels were removed within each SEM image using a FFT band pass filter followed by a threshold adjustment function. The diameter of each particle was then calculated based on the area which was recorded automatically using an analyse particle process.

2.7. Transmission electron microscopy

The presence of NPs in the food simulants following contact with the Ag and Cu nanodot coatings was determined using transmission electron microscopy (FEI Tecnai F30 transmission electron microscope, Oregon, USA). A 5 µl aliquot of food simulant was isolated following migration studies and dried on copper grids (200 mesh copper grid, Electron Microscopy Sciences, USA) for analysis.

2.8. Determination of partition coefficients

The partition coefficient of AgNP and CuNPs between the substrate and food simulant were calculated using Eq. (1).

$$k_{i,P/F} = \frac{C_{i,Peq}}{C_{i,Feq}} \quad (1)$$

where $C_{i,Feq}$ and $C_{i,Peq}$ are the equilibrium concentrations of migrant i in the food simulant and packaging, respectively. It was assumed that equilibrium migration had occurred at the time and temperature conditions used. Therefore, the equilibrium concentration of Ag and Cu in 3% HAc was reached after 10 days at 60 °C. The equilibrium concentration in the coating was then calculated as the difference between the initial concentration of Ag and Cu in the coating, and concentration in the food simulant after 10 days.

2.9. Human exposure

Following migration experiments, it is important to assess the potential human exposure from consumption of migrant Ag and Cu to determine the potential level of risk posed to consumers. Due to absence of NPs in the food simulant, the potential human exposure was determined for ionic species of Ag and Cu. A probabilistic human exposure model was generated in the risk analysis software @Risk (@Risk 6.3, Palisade). To predict human exposure a number of distributions shown in Table 2 were used as inputs to Eq. (2).

$$E_i = \frac{(m_i \times c_{yr})}{bw_{yr}} \quad (2)$$

where “ E ” (mg/kg_{bw}/day) is the human exposure for each of the food packaging additives “ i ” (Ag and Cu) and “ m ” (mg/kg) is the quantity of migrant Ag and Cu determined from the migration studies. The distributions for the amount of fruit juice consumed “ c ” (kg/day) and body weight “ bw ” (kg) are based on different age groups in the Irish population “ yr ” which were sourced from a number of surveys carried out by the Irish Universities Nutrition Alliance (IUNA, 2005, 2006, 2011a & 2011b).

Although there is limited data on the use of NPs in FCMs, fruit juice was used as an example where the nanosilver or nanocopper coating could be applied to impart its antimicrobial activity. The predictive model involving Monte-Carlo simulation was run for 10,000 iterations to account for uncertainty and variability in the input parameters. For comparison of the predicted human exposure to toxicity data, no observable adverse effects levels (NOAEL) for Ag⁺ and Cu⁺ were divided by a species “sp” specific adjustment factor “AF” (IPCS, 2001) shown in Eq. (3).

$$AF_{sp} = IDF_{sp} \times HVF \times UUF \quad (3)$$

This comprises of an interspecies difference factor “IDF” adapted from a study by Bokkers and Slob (2007), a human variability factor “HVF” of 10 and universal uncertainty factor “UUF” of 3 (O’Brien and Cummins, 2010). The NOAEL for Ag⁺ and Cu⁺ orally administered to rats in sub-acute repeated dose studies were adapted from a European Chemical Agency database (Cu⁺ NOAEL of 1000 mg/kg_{bw}/day (ECHA, 2016a) and AgNO₃ NOAEL between 30 and 250 mg/kg_{bw}/day fit using a uniform distribution (ECHA, 2016b)). A margin of exposure (MOE) was then estimated for each scenario by dividing the NOAEL by the predicted human exposure value.

2.10. Statistical analysis

All statistical analysis was carried out in the StatTool 6.3 program (Palisade, Middlesex, U.K.). Differences in size distributions between the AgNP and CuNP samples found by NTA and SEM were tested using a two sample t -test. Differences between Ag and Cu quantities found in the food simulants and food packaging coatings

Table 1
Nanosight settings.

Hardware and software settings	values
Syringe pump flow rates	
Cleaning	1999
sample uptake	50
Capture settings	
No. of captures	15
Duration of captures	60 s
Temperature	25 ± 1 °C
Camera level	14
Screen gain	1
NTA 3.1 analysis settings	
Detection threshold	5
Minimum track length	10 frames

Table 2
Input parameters for human exposure model to Ag⁺ and Cu⁺ in fruit juice (Mean values).

Age bins	c (lognormal)		bw (normal)		m _{Cu} (lognormal) mg/kg	m _{Ag} (lognormal) mg/kg	Reference
	Male kg/day	Female kg/day	Male kg	Female kg			
1	0.023		11.9		0.781	1.374	(IUNA, 2011b)
2	0.038		14.2				(IUNA, 2011b)
3	0.0652		16.7				(IUNA, 2011b)
4	0.0772		18.0				(IUNA, 2011b)
5–8	0.081	0.105	25.5	25.9			(IUNA, 2005)
9–12	0.0874	0.101	39.0	41.6			(IUNA, 2005)
13–14	0.0832	0.085	57.1	55.0			(IUNA, 2006)
15–17	0.09	0.086	65.9	58.9			(IUNA, 2006)
18–35	0.07	0.053	82.5	67.4			(IUNA, 2011a)
36–50	0.047	0.04	88.2	70.5			(IUNA, 2011a)
51–64	0.045	0.032	90.7	73.6			(IUNA, 2011a)
65+	0.043	0.049	82.4	68.1			(IUNA, 2011a)

by ICP-AES, were tested using a two sample *t*-test. To test for differences between particle concentrations found in the food simulants, (which made contact with the nanocoatings), and reagent blank (as measured by the NTA), an ANOVA statistical test was conducted.

3. Results and discussion

3.1. Metal concentration in packaging coating

The total Ag and Cu concentration in the NP coating was determined by complete thermally assisted acid digestion of the coating off the glass substrate followed by ICP-AES analysis (Table 3). The ICP-AES is unable to discriminate between ionic metal and NPs. Therefore it was only possible to present the total Ag and Cu levels in each coating sample. On inspection of the resulting concentrations, there was no significant difference ($P > 0.05$) found between the initial concentration of the AgNP coated slides compared to the CuNP coated slides. It is postulated that the similarity between coating concentrations is as a result of metal saturation which occurs during the coating process.

3.2. Migration levels

Following migration studies carried out according to European Commission (2011) Regulation No. 10/2011 the migration of Ag and Cu nanodot from the BCP PS-b-PEO coating into 3% HAC was found to exceed the migration limit of 0.01 mg/kg for unauthorised substances (Table 3). Despite the high total Ag and Cu migration observed, a notable finding was the significantly lower ($P < 0.05$) migration of Ag observed for the Ag nanodot BCP PS-b-PEO coating. This finding is supported by the higher partition coefficient calculated for the AgNP/PS-b-PEO coating (Table 3). In a study by Liu and Rempel (1991), similar sorption behaviour was experienced for both bulk Ag and Cu on a mercaptoacetamide of aminophenol resin duolite A-7 (MARD A-7) substrate. The results also indicated that Cu was found to leach in diluted HCl, while sorbed Ag was found to be difficultly removed by stronger NH₄OH and HNO₃ solutions. The improved stripping of Ag in HNO₃ may have been accelerated by

decomposition of the resin by the more aggressive acidic solution. The significantly better attachment experienced by the Ag sorbed on the MARD A-7 substrate supports the findings in this study. Another study by Jaiswal et al. (2012) which investigated the release of Ag, Cu and Zinc (Zn) from a sol-gel surface found that Ag had higher migration into distilled water than Cu and Zn. The authors proposed that the reason for this finding is due to the presence of AgNPs in the Ag/sol-gel coating, which had a heightened ability to dissolve in the liquid media when compared to micro-particles and bulk Cu and Zn in the other coatings. In this study, it can be seen from the SEM images (Fig. 1) that there are smaller NPs present in the Cu/PS-b-PEO samples when compared to the Ag/PS-b-PEO samples. The size dependent increase in migration that was experienced by the Ag/sol-gel coating may give justification for the migration levels observed for Cu in this study (Table 3). The smaller Cu particles would cause a larger available surface area for dissolution and consequently cause higher migration levels.

Considering the high migration observed in this study, it is important to note that there is potential to reduce migration by reducing the quantities of Ag and Cu included in the coating during the coating process and to improve the attachment mechanism of the antimicrobial NPs. A recent study by Azlin-Hasim et al. (2015) suggested that sufficient antimicrobial efficacy could be achieved at lower AgNP concentrations within the AgNP/PS-b-PEO coating, than the excessive loading used in the study.

3.3. Scanning electron microscopy of NP coatings

Scanning electron microscopy was used to confirm the presence of NPs in both the AgNP and CuNP coated glass slides (Fig. 2).

There was a significant difference ($P < 0.0001$) found between the size distributions observed for both AgNP and CuNP samples. On the AgNP coated slides well dispersed nanodots were observed on the coating, having 82% of particles counted ($n = 1155$, 12 locations) in the nano range. On inspection of the CuNP coating surface, two distinct populations could be identified (Fig. 1). Particles in the nano size range were present as nanodots, where sub-micron particles formed four cornered star shapes. The high concentration of small particles made it difficult to extract the size

Table 3
Migration and partitioning of Ag and Cu from the nanocoated samples.

	Migration [mg/kg _{food}]	SD [–]	Initial concentration [mg/kg _{food}] ^a	SD [–]	K _{i,FP}
0.6% Ag	0.46	0.19	1.41	0.29	2.18
0.6% Cu ^b	0.82	0.08	1.06	0.22	0.30

^a Initial concentration values are converted for comparison to migration study results (diluent [mg/l] × 0.1[l] × 16 × 6).

^b Mean value is based on 3 replicates.

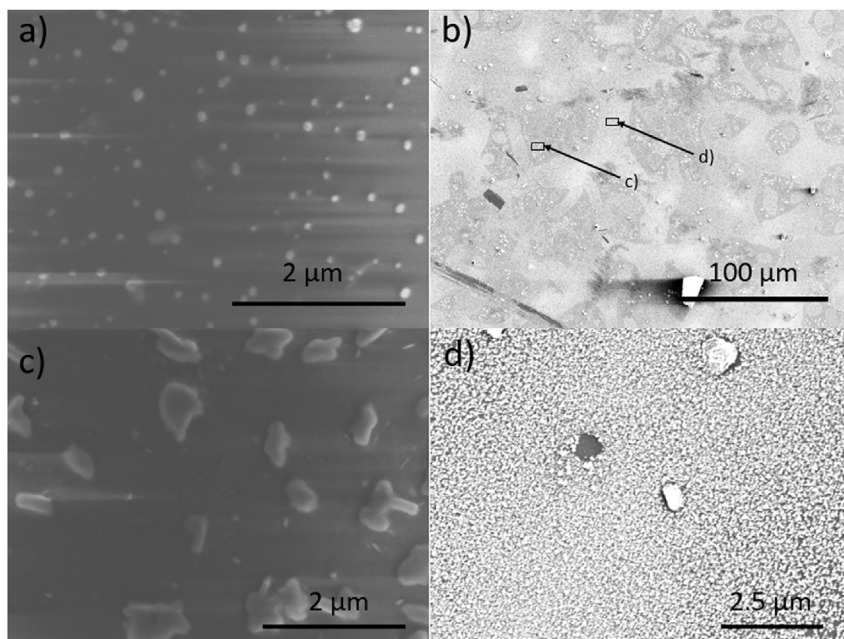


Fig. 1. SEM images of a) AgNP/PS-b-PEO, b) CuNP/PS-b-PEO coating (low magnification showing regions c) and d)), c) large Cu particle region (high magnification) and d) CuNP region (high magnification).

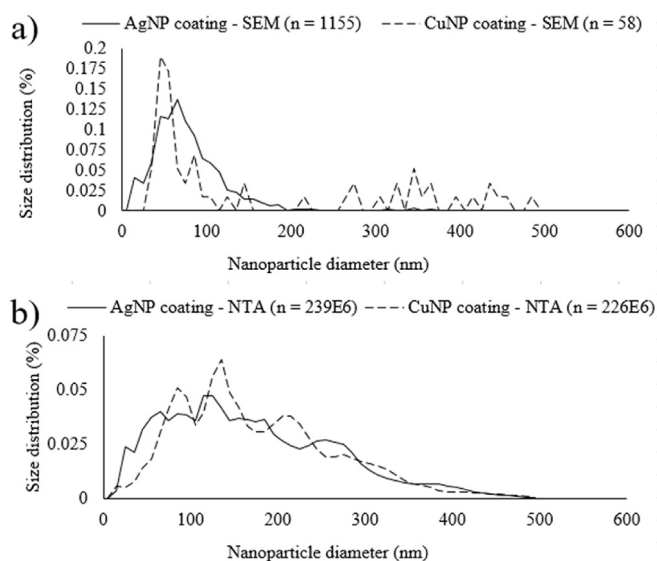


Fig. 2. Size distributions of AgNPs and CuNPs in the nanocoated substrate and food simulant obtained by a) SEM and b) NTA.

measurements from the SEM images so that a reliable size distribution for the coating could be achieved. As a result, there is uncertainty related to the actual particle size distribution for the CuNP coating. Comprehensive characterisation of the NP coatings using X-ray diffraction, atomic force microscopy and energy dispersive X-ray analysis was carried out by [Azlin-Hasim et al. \(2015\)](#).

3.4. Nanoparticle tracking analysis of food simulant

The use of NTA to characterise and quantify the size and particle number concentration in the food simulants has significant advantages over traditional electron microscopy techniques. The high throughput enabled by the NTA's syringe pump increased the

number of particles counted, substantially reducing the analysis time required to gain statistically reliable size distributions. With this said, the size distributions acquired for the AgNPs and CuNPs present in 3% acetic acid were not significantly different ($P > 0.05$) from each other or from the size distributions observed in the reagent blank ($P > 0.05$) suggesting the absence of AgNPs and CuNPs in the food simulant ([Table 4](#)).

The absence of AgNPs and CuNPs in the 3% HAC is more evident when comparing the size-intensity-frequency distributions to those obtained for a 100 nm AgNP standard and the blank 3% HAC simulant using NTA analysis ([Fig. 3](#)).

The absence of AgNPs and CuNPs in the food simulant was confirmed by TEM and SEM-EDX analysis ([Supplementary information 1](#)). When combining this finding with the high total migration quantities observed by ICP-AES analysis ([Table 3](#)) a conclusion related to the migration mechanisms in the system can be drawn. The high levels of Ag and Cu migration and low NP levels in the food simulants would suggest that the main migration mechanism is dissolution of NPs from the packaging surface into metal ions which migrate into the food simulant. Another possible scenario would include the desorption of particles from the packaging surface into the food simulant and rapid dissolution of the NPs into ions. Therefore, the most significant finding is the absence of both AgNPs and CuNPs in the food simulants. This finding is in agreement with a recent study by [Bott et al. \(2014\)](#), in which a stability test of 10 nm AgNPs in ultra-pure water and 3% HAC demonstrated the poor stability of AgNPs in food simulants at a shorter time of 24 h. The signal observed by asymmetric flow-field fractionation (AF4) of the AgNPs was found to reduce in ultra-pure water after 24 h and disappear in 3% HAC after 24 h entirely. In another study by [Mwilu et al. \(2013\)](#), AgNPs were found to quickly dissolve in synthetic stomach fluid (SSF) (deionized water and 0.4 M glycine adjusted to pH 1.6 with 0.42 M HCl) after 30 min. After this period, only 20% of the NPs remained in the SSF. These findings suggest that the test medium pH has a significant effect on the NP stability.

In this study, the AgNP concentration was reduced below the

Table 4
Particle concentration and size as measured by NTA.

	Concentration		Mean (nm)	Mode (nm)	SD (nm)	D10 (nm)	D50 (nm)	D90 (nm)
	Particle/frame	Particle/ml						
Blank	10.6	2.10E+08	210.8	128.5	108.3	71.4	190.8	348.5
0.6% Ag	12.25	2.41E+08	174.8	124.6	98.5	50.3	149.75	299.8
0.6% Cu	11.55	2.28E+08	178.05	128.15	91.9	62.5	152	292.2

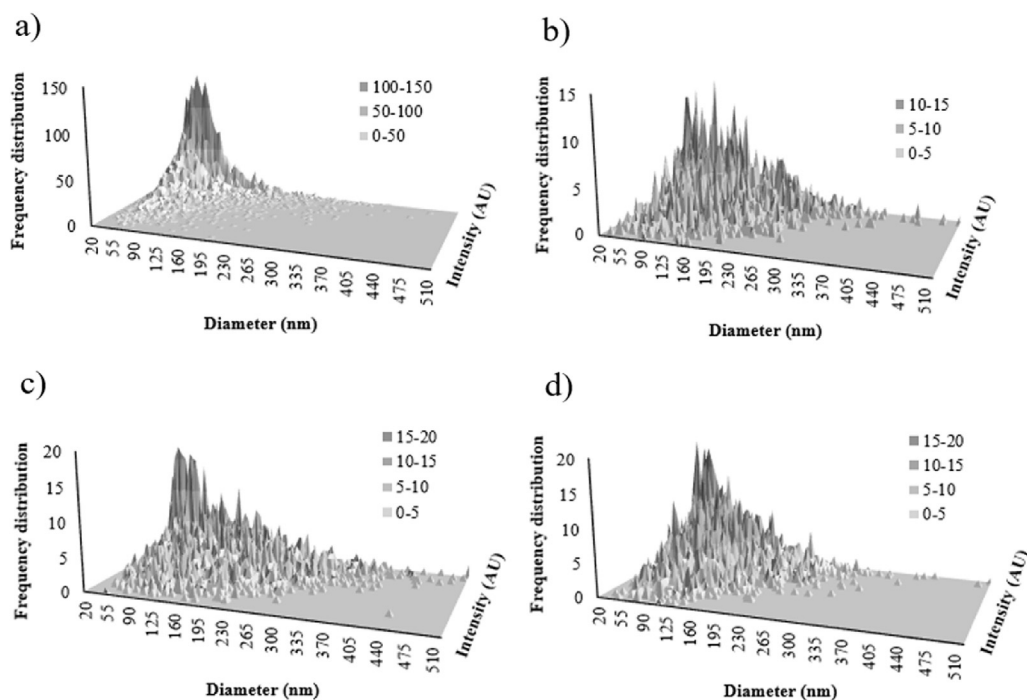


Fig. 3. Diameter-intensity-frequency distribution plots for a) 100 nm standard, b) 3% HAC blank, c) 3% HAC which contacted AgNP/PS-b-PEO coating and c) 3% HAC which contacted CuNP/PS-b-PEO coating.

detection limit of the NTA due to the aggressive nature of the 3% HAC food simulant. As a result, the human risk was quantified based on exposure to Ag⁺ and Cu⁺, and not nanosilver and nanocopper.

3.5. Human exposure

When considering the projected future use of nanotechnology in the food packaging sector, it is important to protect human health by including human exposure assessments to investigate any potential adverse health effects which may be caused as a result of exposure to novel NP products. In this study, the migration of Ag and Cu was found to exceed conservative regulatory migration limits outlined in European Commission (2011) Reg. No. 10/2011. Based on the experimental migration, a human exposure assessment was carried out for both NP coated materials based on the hypothetical scenario that the antimicrobial coated material is used to contain fruit juice (Fig. 4).

Fruit juice was chosen as a potential application for the NP coating as it has the potential for shelf life extension *via* the antibacterial function of the NP coatings. Other products could be packaged using the NP coating that encounter microbial contamination such as; milk and ice cream. In such cases, the improved food safety provided by the reduced microbial populations could merit the use of such coatings. The assumption that Ag and Cu were in ionic form was made based on the absence of NPs in the food simulants observed by the NTA technique. The total transformation

of NPs in the food simulant into ionic species in this study is a good example of case 3 (“Complete ENM transformation in the food/feed matrix before ingestion”) outlined in section 5.2 of a guidance document by the European Food Safety Authority (EFSA, 2011) on the risk assessment of nanotechnologies in food and the feed chain. Based on the predicted human exposure and available toxicity studies, the calculated MOE ranged from 1545 to 29,066 and from 448 to 8499 for Cu and Ag exposure, respectively. Using the commonly used adjustment factor of 100, the MOE ranged from 2043 to 38,451 and from 7114 to 134,516 for Cu and Ag exposure, respectively. These MOE values indicate a low level of concern to adult health when the nanocoating is used in the hypothetical scenario to package fruit juice as the MOE are above 10,000 (EFSA, 2005; Jacobs et al., 2015). The MOE falls below 10,000 for the age groups 2–17 years and 3–8 years for Ag and Cu, however, the MOE are still significantly above 100, which the UK Food Standards Agency (2010) refers to as an acceptable level. An MOE below 1 would be indicative of risk, as the predicted exposure would exceed the NOAEL. It must be noted that the MOE values are based on a conservative adjustment factor incorporating interspecies differences, human variability and uncertainty, subsequently reducing the predicted MOE values. On inspection of the predicted MOE values, it can be deduced that in the given scenario, Cu has the least potential to cause adverse toxicological effects. However, when choosing the most suitable nanomaterial for this antimicrobial application, other factors would have to be considered such as

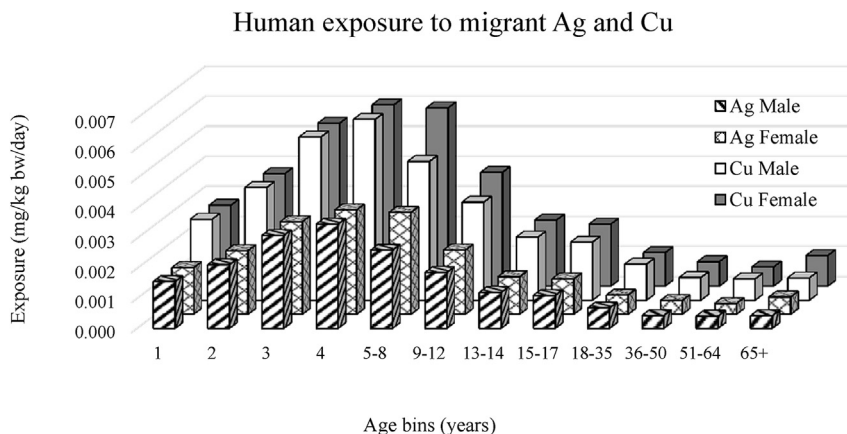


Fig. 4. Predicted human exposure from consumption of Ag and Cu from NP/PS-b-PEO packaging coating.

antimicrobial efficacy. For this application, the superior antimicrobial efficacy of AgNPs (Quirós et al., 2015; Ruparelia et al., 2008) could potentially justify its use instead of CuNPs despite AgNPs lower MOE compared to CuNPs. These findings related to the MOE values are further confirmed when comparing the Ag and Cu exposure values obtained to exposure limits and reference doses found in the literature. In all cases, Ag exposure (0.00021–0.002 mg/kg_{bw}/day) was found to fall below the reference dose of 0.005 mg/kg_{bw}/day established by Faust (1992). It is important to note that this reference dose is based on a lifetime exposure to silver causing argyria, which is an adverse cosmetic effect (Garbos and Swiecicka, 2013). For copper, although the human exposure is higher (0.00038–0.0035 mg/kg_{bw}/day) than silver, the acceptable daily intakes found in the literature for Cu are far higher. The World Health Organisation (1996) established an acceptable copper intake of 10–12 mg/person/day with a natural intake of 1–2 mg/person/day. Based on this data, the European Food Safety Authority (2008) concluded that an acceptable upper exposure limit was 0.15 mg/kg_{bw}/day, with exposure limits of 0.2 mg/kg_{bw}/day and 0.15 mg/kg_{bw}/day for adults and children, respectively. On inspection of the calculated Ag and Cu exposure levels from the AgNP/PS-b-PEO and CuNP/PS-b-PEO coatings, both levels were below the exposure limits found in the literature signalling the potential use of these NP coatings to package fruit juice. An important outcome from both the experimental migration study and subsequent human exposure assessment is the discrepancy between the satisfactory safety factor (MOE) calculated for both materials and migration which was found to exceed regulatory migration limits for unauthorised substances from food contact materials (European Commission, 2011). These findings suggest that current migration regulations for unauthorised substances are conservative for these materials under the conditions assessed in this study and future amendments could include minimum safety factor conditions for human exposure assessments. There is uncertainty that surrounds the most suitable risk orientated criterion for the safety assessment of such nanomaterials, whether it be migration, human exposure or MOE. In European Commission (EU) regulation No. 10/2011, specific migration limits are formulated using toxicological data such as the NOAEL incorporating safety margins ranging from 100 to 1000 and including an assumed human exposure, e.g. 1 kg food packaged in 6 dm² consumed by an individual of average weight (Piringer and Baner, 2008). The MOE is a risk management tool that gives an indication of the level of human health concern, which incorporates the human exposure for a specific scenario, toxicity data and an adjustment factor to account for uncertainty and variability. In this case, ionic Ag and Cu

were evaluated in terms of migration, human exposure and MOE due to the absence of NPs in the food simulant. In the event that NPs persisted in the food simulant, the MOE would have to include both toxicity and human exposure quantities in terms of other dose metrics (i.e. m²/kg_{food} or particles/kg_{food}) specific to the NPs in question. It is recognised that for both AgNPs and CuNPs there is a lack of suitable toxicity studies providing this information, making it difficult to enable such evaluation (Cushen et al., 2014). With regard to the calculated MOE values, it is important to note that this study uses the worst case conditions for migration, as well as high concentrations of Ag and Cu in the coating to give a higher antimicrobial activity. As a result, there is potential to improve the MOE by reducing the concentration of Ag and Cu in the nanocoating or alternatively choosing an application for the nanocoated food packaging that would significantly reduce migration (e.g. dry food product).

4. Conclusions

This study investigated the human exposure to Ag and Cu migration from a nanosilver and nanocopper FCM surface coating used in the hypothetical scenario as fruit juice packaging. To examine the potential migration of AgNPs and CuNPs a suite of techniques were used which included; SEM, TEM, ICP-AES and NTA. Following accelerated migration studies at 60 °C for 10 days in 3% HAC, the level of total Ag and Cu migration into a food simulant was observed by ICP-AES. Migration levels were found to exceed the European Commission regulatory limit for unauthorised substances of 0.01 mg/kg. A noteworthy finding is the lower migration observed for the AgNP/PS-b-PEO coating. It is thought that this may be due to the increased dissolution rate of the CuNPs as a result of their smaller size in the CuNP/PS-b-PEO coating in comparison to the AgNPs size in the AgNP/PS-b-PEO coating. The increased size-dependent dissolution of CuNPs would in turn cause higher migration into the 3% HAC than AgNPs. Despite the high total migration observed, there was no significant difference between particle concentrations found in the food simulant which had been in contact with the AgNP and CuNP coatings, and the reagent blank measured by NTA. The absence of NPs in the food simulant was confirmed by TEM. This would suggest that the main mechanism for migration is dissolution of NPs from the packaging coating, or particle desorption and then rapid dissolution in the acidic food simulant. Similar behaviour supporting the poor stability of AgNPs has been reported in the literature. Human exposure was quantified for ionic species of Ag and Cu that were present in the food simulant. Using the predicted human exposure from this study and

toxicity data available in the literature, MOE values were calculated for Ag and Cu exposure from the nanocoatings. MOE values ranged from 1545 to 29,066 and from 448 to 8499 for ionic Cu and Ag exposure, respectively. For both materials, the MOE calculated for each scenario was above 100 which indicates an acceptable level of concern and a lower level of concern in a number of scenarios (MOE > 10,000) when used in the hypothetical scenario as fruit juice packaging. On inspection of the MOE values, the CuNP coating was found to present the highest MOE in the given scenario. In light of this statement, other factors such as antimicrobial efficacy would also impact on the most suitable choice of coating for packaging applications. It must be noted that both the experimental parameters and NP inclusion in the coating favoured migration. Therefore, there is potential to improve the MOE value by reducing the NP inclusion levels, improving attachment or by choosing applications for the food packaging coating which would inhibit migration.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.fct.2016.07.004>.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.fct.2016.07.004>.

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Conflict of interest

The authors declare that they have no conflict of interest.

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