Application and safety assessment for nano-composite materials in food packaging

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The technical breakthroughs and rapid developments in nanotechnologies enabling goods and products based on nanomaterials (NMs) has come to media and public attention as potentially one of the most significant technological advances of our time. Among these technical applications, nano-composite food packaging, which combines the NMs and conventional packaging, is at the forefront of applications in nanotechnologies, leading the whole industrial chain based on nanotechnologies with high speed development. Taking into account the status of the nano-composites applied in food packaging and present development trends, this review is focused on summarizing the basic applications of the nano-composites in food packaging as well as its risk assessment. The status summaries and the conclusions derived from this review are of benefit to manufacturers which produce the nano-composites used in food packaging, to general consumers and to governmental administration entities.

nanometerials, composites, nano-composite materials, food packaging, safety assessment

Nanotechnology has been the subject of rapid expansion in the development and application of food packaging in recent years. In the process of food manufacturing, the additive surface functional nanoparticles (NPs), which are characterized by small size (< 100 nm), large surface area, and a high surface reactive property, can significantly improve food flavor, color and texture. They also increase nutrition absorption and food bioavailability, and bring human health benefits [1–4]. In the area of food packaging, the nano-composite, which combines the traditional food packaging materials with the NMs, has been subject to high speed development in the food packaging market with its excellent mechanical performance and good antibacterial and stronger resistant properties [5]. Reviewing the whole food manufacturing chain, the nanotechnologies used in food processing are more abundant than those in food packaging. The reason for this status is that most people are dubious about the use of NMs before its safety is confirmed by reliable and convincing theoretical assessment and experimental data. In addition, from a technical viewpoint, the manufacturing methods and skills in combining NMs into packaging materials, such as polymers, paper and paperboard, glass and ceramic, and the surface of metals, are well developed. This is particularly true when compared with the current level of manufacturing methods and skills applied in nanostructured (or nanotextured) food ingredients, the delivery system for nutrients and supplements, and the nano-encapsulations of food ingredients and additives.

According to the market study report accomplished by Helmut Kaiser Consultancy in 2007, the type of the nano-composites food packaging products expanded from less than 40 in 2002 to more than 400 in 2006. In the next decade nanotechnology will impact 25% of the food packaging market, which is currently estimated at $100 billion

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(www.foodproductiondaily.com/Packaging/Future-nanopackaging-market-worth-billions-says-study). The reason for this is that the general public thought the use of “fixed” NPs in food packaging materials was relatively safe and would not affect the quality of packed food. However, theoretical and experimental data simultaneously demonstrated that the NP composites used in packaging had the potential and tendency to migrate to food [6]. Also, the scientific reports conducted by the United States, European Union (EU), Japan, South Korea and China indicated that NMs have health and environment toxicities, which are dependent on its physicochemical characteristics such as size, morphology, and surface properties [7]. These raised public concern about NMs. As a result, the 6th Meeting of REACH in Brussels in December 2008 reached the conclusion that NMs were industrial materials and has taken them into its range of supervision.

This review summarizes the research literature, scientific reports, and resources of the internet on the applications of NMs in food packaging. Based on the results, a safety assessment for the use of NMs in food packaging is developed. This review is beneficial to the manufacturers which produce nano-composites used in food packaging, to general consumers and to governmental administration and supervision entities.

1 Applications in food packaging

According to laboratory research, market-oriented applications and development trends, three main categories of nanotechnology are used in food packaging applications: NPs-reinforced packaging, active and intelligent packaging, and biodegradable nano-composites food packaging.

1.1 NPs-reinforced packaging

The high surface area enables NPs to dramatically improve the mechanical performance, such as flexibility, reduced gaseous permeation, stability of temperature and humidity, and ultraviolet light and flame resistance, of packaging materials with a relatively low mass content (~5%, w/w). In 2001, nano-composites constructed by polymer and nano-sized montmorillonite (MMT) have been shown to improve the thermal-resistance properties of packaging materials [9]; composites made of polyactic acid (PLA) and nano-sized MMT could improve the fire-resistance [10]; polyethylene (PE)-MMT and PE-silica (SiO₂) composites improved durability [11]; a nano-composite synthesized by polyvinyl chloride (PVC) and MMT could improve the property of optical resistance [12]. A novel composite prepared by polyamide and multiwall carbon nanotube (MWNT) offered significant flame-resistant properties [13]; another composite composed of polymer and nano-sized MMT improved gas barrier properties [14]. The variety of NP-reinforced polymers has attracted increasing attention, and nano-composites use in food packaging has become the most developed area in nanotechnology [15].

Apart from improving mechanical properties, the application of NPs can bring extra functions, such as an antibacterial property, to food packaging. Silver (Ag) NPs are the most frequently used material for the purpose of providing an antibacterial property [16–18]. Ag NPs used in packaging or food contact materials can prolong foodstuff shelf life by restraining the growth of bacteria. For example, the NPs-polymer composites can form coatings on the surface of tableware and kitchenware to inhibit the growth of microorganisms. Examples of this type of tableware and kitchenware are found in products made by Nano Care Technology in China (www.nanocaretech.com/En_ArticleShow.asp?ArticleID=13) and A-DO in South Korea (www.adox.info/?doc=shop/list.php&ca_id=150110). Recently, researchers from the University of Leeds, UK, discovered the antimicrobial properties of nano-zinc oxide (ZnO) and magnesium oxide (MgO). Compared with Ag NPs, the ZnO and MgO NPs are expected to provide not only more affordable and safe food packaging solutions in the future, but also a greater ability to inhibit the growth of microorganisms (www.foodproductiondaily.com/Packaging/Nanotechnology—discovery-promises-safer-food-packaging). An example is SongSing Nano Technology Co. Ltd., which introduced the oxide-based light catalyst ZnO NPs into their plastic wrap, which claimed to sterilize under indoor lighting (www.nanotechproject.org/inventories/consumer/browse/products/nano_plastic_-wrap/). More examples for Ag and ZnO NPs used in food contact materials are summarized in Table 1, with products manufactured by large companies such as Bayer and Honeywell.

1.2 Active and intelligent food packaging

The active and intelligent food packaging category is a novel type of packaging compared with traditional methods. In active and intelligent food packaging, the “active” refers to the packaging which has the ability to remove undesirable tastes and flavor, and improve the color or smell of the packed food. For example, the incorporated carbon black (CB) NPs and MWNT in polymer packaging can absorb the smell emitted from the packaging or food; furthermore, an incorporated spice can preserve the color and smell of the food [19–21].

“Intelligent” food packaging seems to have a broader application than “active” packaging. In general, the “intelligent” aspect of food packaging refers to the concept of monitoring information about the quality of the packed food. For instance, packaging materials incorporating nano-sensors or nano-capsules based on nanotechnology will be able to detect food spoilage organisms and trigger a color change to alert the consumer that the shelf life is ending or has ended. This type of function can also incorporate
Table 1  Selected market products with NP-reinforced properties

| Manufacturer                                    | Application & properties                                      |
|-------------------------------------------------|-------------------------------------------------------------|
| SongSing Nano Technology                        | Food cling wrap treated with ZnO NPs; tea pot treated with Ag NPs |
| Sharper Image and BlueMoonGoods                 | FresherLonger™ Miracle Food Storage Containers and Plastic Storage Bags |
| Daewoo, Samsung, LG and Midea                   | Ag NPs incorporated into the inner surface of domestic refrigerators |
| Baby Dream                                      | Ag NPs baby milk bottle                                     |
| A-DO Global                                     | Ag NP-coated cutting board                                  |
| Nanocor                                         | Branded with Imperm®. Used in multi-layer PET bottles for food and beverage packaging to minimize the loss of CO2 from the drink and the ingress of O2 into the bottle |
| Honeywell                                       | Branded with Aegis® OX. Resin composed of nylon and MMT NPs, enhance barrier properties for retaining CO2 and keeping O2 out. Used in PET bottles such as beer, fruit juice and soft drinks |
| Bayer                                           | Branded with Durethan® KU2-2601. Hybrid plastic comprises polyamide and layered silicate barriers. The plastic incorporates Nanocor’s MMT to produce a film with increased barrier properties, enhanced gloss and stiffness |

A concept of “Release-on-Command”, which will provide a basis for intelligent preservative packaging technology that will release a preservative if food begins to spoil (www.azonan-o.com/Details.asp?ArticleID=1317). In addition, packaging containing sensor arrays is another novel technique based on the concept of an “Electronic Tongue”, which can monitor and signal the condition of the packed food [22]. More examples based on active and intelligent techniques are summarized in Table 2.

Among the new-market developments are NMs-based next generation packaging displays that include Radio Frequency Identification Display (RFID). These displays involve utilization of smart labels that will assist quick and accurate distribution of a wide variety of goods with limited shelf-life. Also under development are RFID’s incorporating polymeric transistors that use nanoscale organic thin-film technology. The RFID systems will be designed to operate automatically and will provide exception reports for anomalies such as temperature, short-life span products.

1.3 Biodegradable nano-composite food packaging

The biodegradable nano-composite food packaging category involves new types of biodegradable materials, which in general can be made of PLA and MMT. MMT is a type of relatively cheap and widely available natural clay derived from volcanic ash/rocks [23–26]. The naturally existing nano-layered structure limits the permeation of gases, and provides substantial improvements in gas barrier properties of the nano-composite. Such improvements have led to the development of PLA-MMT nano-composites for potential use in a variety of food-packaging applications, such as processed meats, cheese, confectionery, cereals, as well as boil-in-the-bag foods. In 2003, a novel nano-composite composed of biodegradable polymer and nano-sized MMT prepared by the McGlashan group offered excellent recycling properties [25]. Other successful applications in the market based on biodegradable nano-composites are summarized in Table 3.

2 Safety assessment for nano-composite food packaging

2.1 Migration study

The main risk of consumer exposure to NPs from food packaging is likely to be through potential migration of NPs into food and drink. However, migration experimental data are not currently available, despite the fact that a number of food packaging types containing NPs are already available and in commercial use in some countries.

The pioneering work was accomplished by a group led by Maurizio Avella from Italy in 2005. Nano-composite

Table 2  Packaging products based on active and intelligent techniques

| Manufacturer or Institution            | Application & properties                                      |
|----------------------------------------|-------------------------------------------------------------|
| CSP Technology                         | Polymer capable of releasing ingredients into the food or beverage in response to external stimuli, to control humidity, oxygen, bacteria, odor and even the flavor of the food itself |
| Kraft                                  | Nano-sensor based “electronic tongue” able to “taste” chemicals to the level of parts per trillion and then guide chemical release, to control the release of smell, taste and nutraceuticals into food products in response to the preferences of individual consumers |
| Georgia Technology                    | MWNT based biosensor, to detect microorganisms, toxic proteins, or spoilage of foods and some beverages |
| University of Southampton, UK & Deutsches Kunststoff-Institut, Germany | “Opal” film, incorporating 50 nm CB NPs, to change colour in response to food spoilage |
| University of Strathclyde, Scotland    | UV-light activated, TiO2 NPs based, oxygen-sensing ink, for the purpose of tamper proofing |
| Australian company MiniFAB            | Nanotechnology based biosensors, to detect biological contamination |
films were obtained by homogeneously dispersing functionalized layered MMT NPs in different thermoplastic starches, such as neat potato starch and a mixture of potato starch with biodegradable polyester, via a polymer melt processing techniques [26]. The conformity of the nano-composites with actual regulations and European directives on biodegradable materials was verified by migration tests. The vegetable samples, including lettuce and spinach, were cut into small samples and packed into bags for the contact-tests. Subsequently the closed bags were placed in an electrical furnace and heated to 40°C for 10 days. After this process, every bag was slowly cooled to the ambient temperature and submitted to subsequent characterization. The vegetable samples were digested by heating in a furnace at 105°C to constant weight, then carbonized in a muffle at 550°C for a few hours and cooled in a dry atmosphere for 40 min. The ash obtained was dissolved in hydrochloride acid solution, put in a hot water bath and finally filtered. The filtrate was analyzed by atomic absorption equipment to measure the Si, Fe and Mg release. The analyzed results demonstrated an insignificant trend in the levels of Fe and Mg in packaged vegetables, but a consistent increase in the amount of Si, which is the main component of the MMT NPs.

A group from the Slovak University of Technology studied the NP migration based on the concept of chemical potential [6]. This study took into account physicochemical properties of both NPs and packaging polymers, such as the density of the polymer, the size of the NPs, the density of NPs in the polymer and the average distance of NPs from the surface of the polymer. They deduced the migratability, eq. (1), of NPs in polymer and the migration numbers, eq. (2), of NPs from the nano-composites making use of Fick’s second law of diffusion:

\[ m = \frac{k_B T \pi}{24 \eta a^2}, \]  
\[ n = mS c_0. \]

In eq. (1), \( m \) is the migratability of NPs in polymer, \( k_B \) is the Boltzmann constant (\( k_B = 1.3807 \times 10^{-23} \text{ J/K} \)), \( a \) is the radius of NPs, \( T \) stands for the absolute temperature, \( t \) stands for the migration time and \( \eta \) stands for the dynamic viscosity of the polymer. It can be seen that the migratability of NPs is controlled by the size of NPs and the density of the polymer matrix. The smaller size of NPs and smaller density of polymer will result in higher migratability. In eq. (2), \( n \) stands for the amount of migrating NPs, \( S \) stands for the surface area of the polymer film and \( c_0 \) is the initial concentration of NPs in the polymer film. By using eqs. (1) and (2), they calculated \( m \) and \( n \) for different NPs with various radii and polymer with different densities. Taking the surface area as 0.2 m² of low density PE (LDPE) and 5 nm Ag NPs as example, provided that the initial concentration of the NPs in LDPE is 1 kg/m³, the calculated migratability at a temperature of 25°C in LDPE is \( 1.3 \times 10^{-6} \), and the amount of the migrating NPs is \( 2.6 \times 10^{-7} \) kg within 1 year. In the case of polyethylene terephthalate (PET), the amount of migrating NPs is less than \( 2.2 \times 10^{-8} \) kg. These theoretical calculations indicated the migration of NPs in nano-composites strongly depends on its size and the density of the matrix.

Migration studies of NPs in food contact materials are relative scarce. The difficulties in characterizing NPs in composites and the lack of methods for qualitative and quantitative analysis are responsible for this limited research on migrating of NPs in nano-composites.

### 2.2 Sources and distribution

According to the scientific report from the European Centre for Ecotoxicology and Toxicology of Chemicals, there are three main routes for human exposure to NPs: inhalation, dermal contact and oral ingestion [27]. The atmosphere within and around workplaces in the manufacturing process is the source of exposure. After inhalation, the NPs will deposit within the respiratory system (mainly the lung) by diffusion. General speaking, the smaller NPs have a higher probability of affecting the epithelium of the lung structure. Insoluble NPs larger than 100 nm, will move, by the action of ciliated cells in the mucus, or by coughing, within 1–2 days, to the throat (larynx), where they are swallowed. However, small NPs will be taken up and digested by the specialized defense cells called macrophages, located in the alveoli. The interaction of NPs with the alveoli will transport NPs from luminal to the mucosal side of epithelial and endothelial cells by the function of vesicular caveolae. Transport within caveolae for small NPs exists across the
alveolar capillary barrier as a pathway from the lung to the blood [28,29]. With the help of blood circulation, NPs will be transported to other systems such as the liver, spleen, kidney, brain and heart.

Uptake by skin contact can occur through three possible penetrating pathways: intercellular, intracellular, and follicular penetration. Among these, follicular penetration is a direct and effective way for NPs to be absorbed by the skin, even though the amount of hair follicles on the skin surface was estimated to be not more than 0.1% of the total skin surface area [30,31]. However, increasingly, studies are revealing that the NPs taken up by the skin remain on the surface of the stratum corneum or accumulate in the follicle orifices, whichever penetration pathway they take, but do not penetrate the skin or the follicle [32,33].

Oral uptake from food with added NPs, and any migrations from the nano-composites, is the most significant exposure source for human beings. The digested NPs will temporarily remain and accumulate in the gastrointestinal (GI) tract, and are then absorbed by the intestinal epithelium to be transported to other systems and organisms. Translocation of NPs through the intestinal wall is a multi-step process, involving diffusion through the mucus lining of the gut wall, contact with enterocytes or M-Cells, cellular or paracellular transport, and post-translocation events [34]. During the translocations, factors such as size, surface charge, lipophilicity/hydrophilicity, presence/absence of a ligand, and physiology of the intestinal tract, e.g., healthy vs. diseased state, will influence the translocation process [35].

Smaller particles are generally absorbed more readily and faster than larger ones. Diffusion across the preepithelial mucus gel layer of the distal colon of rats was conducted by Szentkuti’s group in 1997, and the results demonstrated that 14 nm (average in diameter) PLA NPs cross within 2 minutes and 415 nm within 30 minutes, while 1000 nm did not cross this barrier [36]. Oral administration of Au-NP, with different sizes of 58, 28, 10 and 4 nm, to mice, showed increased GI uptake with diminishing size [37]. The amount of absorption of polystyrene (PS) NPs of 50, 100, 200, 300, 1000 and 3000 nm has been shown to be 34% in rats at 50 nm, and decreasing to almost zero with increasing size [38]. Passage of NPs through the intestinal mucus is not only dependant on the size, but also the surface charge. Furthermore, experiments by Szentkuti, with different charges difused through intestinal mucus, showed that the cationic NPs were found to become entrapped within the negatively charged mucus, whereas carboxylated (anionic) NPs were able to diffuse successfully through to the epithelial surface [36]. Very recently, the researchers at John Hopkins University in Maryland, USA, discovered that pores within the mucus layer are much larger than originally anticipated, and NPs as large as 200 nm can pass through mucus pores when coated with polyethylene glycol (PEG) [39].

Once NPs have diffused through the gut mucosa and been in contact with enterocytes and M-cells, they will pass through the epithelia. There are several mechanisms by which NPs may pass through the epithelia: transcytosis by enterocytes, transcytosis by M-cells in Peyers’s patches (PP) and passive diffusion across the epithilia or paracellular transport. The GI uptake rate of NPs is 2–200 times greater in PP than in enterocytes even though the PP represent only ~1% of the total intestinal surface area [35]. The translocation of NPs in the epithilium is also dependant on their size. For example, translocation of NPs with an average size of 116±5 nm is 15–250 times greater than that of microparticles, which are more likely to become lodged within the PP [35]. The GI absorption of NPs may be affected by different surface coatings and zeta potential.

Upon contact with the intestinal submucosal tissue, NPs can enter the capillaries, which will carry them through the portal circulation to the liver, or they enter the lymphatic system, which via the thoracic duct empties into the blood circulation system. In a 28-day rat oral study of 60 nm Ag-NPs, the highest Ag levels occurred in the stomach, followed by kidney and liver, lungs, testes, brain and blood [40]. The distribution is dependent upon the size of the NPs. In the administration of Au-NP sized 58, 28, 10 and 4 nm to mice, the smaller particle size resulted in increased distribution to the organs. The smallest NPs were found in the kidney, liver, spleen, lungs and brain, while the biggest remained almost solely inside the GI tract [37]. PS NPs ranging from 50 nm to 3 μm were fed by gavage to female Sprague Dawley rats daily for 10 days at a dose of 1.25 mg/kg\(^{-1}\). The extent of absorption of the 50 nm particles was 34% and of the 100 nm particles 26%, of which about 7% (50 nm) and 4% (100 nm), was in the liver, spleen, blood and bone marrow.

2.3 Toxicity

The migration of NPs from packaging to packed food raised public concern, and had been corroborated by animal oral administration and in-vitro cell experiments. In general, two types of mechanism can be adopted to explain the toxicity effects on humans. One is that the toxicity is independent of the NPs, and could be realized by generating the active oxygen species (ROS) within the cells. Another is that the toxicity has a strong relationship with the chemical component of NPs. For example, the crystallization and recrystallization of some metal or metal oxide NPs will modify the secondary or tertiary conformation of the proteins. While other types of NPs, such as the metal alloy, or single-wall carbon nanotube (SWNT) or MWNT can result in direct or indirect gene toxicity.

In vitro experiments have shown that the NPs within cells will generate a large number of ROS. It is well known that shrinkage in a number of materials is responsible for the change in the physicochemical and electron structural of some NPs. For instance, the decrease in size of bulk materials
may create discontinuous crystal planes and enable more atoms within the materials to be at the surface of the NPs, which increases the number of structural defects and dangling bonds as well as disrupting the well-structured electronic configuration of the materials, giving rise to altered electronic properties [41,42]. The altered electronic structure establishes specific surface groups that could function as reactive sites. For instance, the altered electronic structures in semiconductor and metal NPs unbind the surface atom and within the electron-hole pair become the reactive sites. Electron capture within the reactive sites with molecular dioxygen (O₂) can lead to the formation of the superoxide radical O₂⁻, which through dismutation or Fenton chemistry can generate additional ROS. The ROS generation is independent of the chemical compositions of NPs, i.e., NPs like the metal, metal alloy, metal oxides, semiconductors and nonmetal CB (including nanotube and fullerene (C₆₀ and C₇₀)) can generate the ROS within the cell. However, in normal conditions, the mitochondria in cells can generate the ROS at low frequency and are easily neutralized by antioxidant defenses such as glutathione (GSH) and antioxidant enzymes [43]. However, the presence of NPs, which will preferentially mobilize the mitochondria in cells, will interfere and alter the ROS production and thereby overload or interfere with the antioxidant defenses. The overwhelmed antioxidant defenses enable the cells to transform into a state in which the GSH is depleted while oxidized glutathione (GSSG) accumulates, which is called oxidative stress [43]. The oxidative stress can lead to a decrease in the ability of cells to form an antioxidant defense, and to the accumulation of GSSG, resulting in respiratory tract inflammation and interstitial fibrosis.

From a mechanistic perspective, ROS generation and oxidative stress is the best developed paradigm to explain the toxic effects of NPs. In 2003, a group lead by Nel in America studied the relationship between mean size, size distribution, and composition of NPs and their ability to induce oxidative stress [44]. The results indicate that the higher the monodispersion of NPs, the higher the volume of ROS will be generated and the stronger the oxidative stress upon the cells. At the same time, the research group led by Yamakoshi from Japan studied the process and mechanism of fullerene (C₆₀ and C₇₀) generating ROS in cells, and quantitatively determined the content of ROS (O₂⁻ and ‘OH) by biochemical (DNA-cleavage assay in the presence of various scavengers of active oxygen species) and physico-chemical (Electron Paramagnetic Resonance (EPR) radical trapping and near-infrared (NIR) spectrometry) means [45]. Oxidative stress can also influence the central nervous system. For example, an experimental study demonstrated that oxidative stress is implicated in the pathogenesis of neurodegenerative diseases such as Parkinson’s and Alzheimer’s [46]. Evidence for such effects is presented by studies on biopsies from city dwellers and Alzheimer-like pathology has demonstrated increased markers of inflammation and AB42-accumulation in the frontal cortex and hippocampus in association with the presence of NPs [47].

Toxicity induced by oral administration will influence the liver and spleen. The reticuloendothelial system located in the liver and spleen have the ability to phagocytose cellular debris, aged cells, pathogens and foreign substances including

| NMs & applications | Size & physical description | Experimental evidence of toxicity |
|--------------------|-----------------------------|----------------------------------|
| TiO₂, used as antimicrobial and UV protector in food packaging | 20 nm 30 nm, mix of rutile and anatase forms NPs, mix of rutile and anatase forms Four sizes 3–20 nm, mix of rutile and anatase form 25 nm, 80 nm, 155 nm 15 nm 15 nm, 100 nm 15 nm 20 nm, 120 nm ZnO powder 19 nm ZnO | Destroyed DNA DNA damage to human skin cells when exposed to UV light High concentrations interfered with the function of skin and lung cells. Anatase particles 100 times more toxic than rutile ones 25 nm and 80 nm particles caused liver and kidney damage in female mice. TiO₂ accumulated in liver, spleen, kidneys and lung tissues Highly toxic to mouse germ-line stem cells Highly toxic to rat liver cells Toxic to rat brain cells 120 nm particles caused dose-effect damage in mice liver, heart and spleen. 20 nm particles damaged liver, spleen and pancreas Toxic to human and rat cells even at very low concentrations Test mice showed severe symptoms of lethargy, vomiting and diarrhea NP dose produced more severe response, killed 2 mice in first week, and caused greater kidney damage and Anemia. Greater liver damage in microparticle treatment 50 nm and 70 nm particles taken up into cell nucleus where they caused aberrant protein formation and inhibited cell growth. Caused the onset of a pathology similar to neurodegenerative disorders |
inert particles from the blood stream. Such cells include macrophages, monocytes, and specialized endothelial cells that line the organs such as the liver, spleen and bone marrow. The main function of this system is thought to be the removal and neutralization of any potential pathogens that enter the body from the GI microflora. However, these cells have the capacity to phagocytose NPs. Evidence from in vitro studies that NPs such as CB and PS stimulate the macrophages via ROS and Ca signaling to make proinflammatory cytokines such as the tumor necrosis factor alpha [48]. Oxidative stress is known to inhibit the hepatocyte function and bile formation [49], while proinflammatory cytokines are also associated with the pathology of liver disease.

NPs through oral administration may create cardiovascular effects, which include heart rate changes, prothrombosis and acute myocardial infarction. For instance, research results derived from experiments led by Samuel on imaging angiogenesis by inter-intargeted paramagnetic iron oxide, demonstrated that cationic NPs, including Au and PS can cause hemolysis and blood clotting, while usually anionic NPs are quite nontoxic. Another experiment conducted by Campen’s group, on hypertensive rats, showed that the rats with no oral administration displayed a reduced daytime heart rate from the beginning of the protocol, whereas exposed rats maintained a significantly elevated heart rate. Daytime heart rate values for male control rats averaged 265 ± 5 beats/min, whereas values for exposed rats averaged 290 ± 7 beats/min [50]. In 2002, a test on the effect of exposure to NPs on the progress of atherosclerosis in rabbits was conducted by a research group from Canada. Results of that experiment indicated that a high dose of NPs in Watanabe rabbits caused morphological evidence of atheromatous plaque destabilization [51]. Ultrafine CB NPs digested into the blood has been reported to induce platelet accumulation in the hepatic microvasculature of healthy mice in association with prothrombotic changes on the endothelial surface of the hepatic microvessels [52]. Although several toxicological studies have demonstrated that the oral administration of NPs can gain access to the blood and can enhance experimental thrombosis, it is not clear whether this is a pulmonary inflammation or NPs translocated to the blood [47].

Some toxicities are dependent on the chemical composition of the NPs. For example, experimental results indicate that the accumulation of insoluble NPs in humans may be responsible for the compromised GI functionality, including irritable bowel disease (IBD) and Crohn’s disease [53]. Although little is known about whether NPs are linked to the initiation of IBD and Crohn’s disease, it seems that the NPs may be adjuvant triggers for the exacerbation of these diseases [54]. For instance, some insoluble NPs, such as TiO$_2$, ZnO and SiO$_2$, when they are absorbed by GI and pass through the intestinal tract, come into contact with and adsorb calcium ions and lipopolysaccharide. The resulting NPs–calcium–lipopolysaccharide conjugates activate both peripheral blood mononuclear cells and intestinal phagocytes, which are usually resistant to stimulation [55]. Metal or metal oxide NPs, such as Fe/Plt alloy, Co/Cr alloy, ZnO, SiO$_2$, TiO$_2$, and SWNT and MWNT, present direct genotoxicity and indirect genotoxicity [56,57]. For TiO$_2$ and CB, it was reported that particles with a mean size of ~20 nm induced DNA damage, while larger particles (~200 nm) did not [56,58,59]. Similar observations were reported for Co-NPs [60]. More research studies on toxicity are summarized in Table 4. From Table 4, one can see that the toxicity of NPs is strongly related to its chemical composition and particle sizes.

### 3 Detection methods

In contrast to techniques used for small organic molecular and inorganic compounds and the low-molecular-weight polymers, the techniques for NPs and NPs in nano-composites are more sophisticated, typically because of the strong relationship between the functions of the packaging materials, and the toxicity morphologies in the packaging matrix. Therefore, apart from the traditional instrumental methods, other special means of determination such as imaging techniques are needed for successful characterization of NPs.

#### 3.1 Imaging techniques

Imaging techniques for detection of NPs are mainly electronic microscope techniques and related facilities, such as transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM). In 2003, Morgan of Dow Chemicals, characterized the MMT NP dispersion in polymers by using TEM. The research nano-composites ranges include thermoset (cytane esters) and thermoplastic polymers (PS, nylon 6, and polypropylene-g-maleic anhydride) [61]. Researchers from the CNRS in France characterized the size and shape of MMT NPs located within the PP-MMT composites made by using two different facilities. A statistical TEM image analysis methodology was developed to evaluate the different particle parameters (thickness, length, aspect ratio and interparticle distance). The results obtained showed a correlation between the size of the tactoids and the shear intensity [62]. In addition, Japanese researchers from Toyota observed the microstructures of the nano-composites of nylon-MMT with the help of SEM. The results revealed a novel structure, in which the layered MMT NPs stood perpendicularly to the nylon surface, resembling a vertical cliff [63]. Perrin-Sarazin group analyzed the shape and morphology of MMT NPs by simultaneously using TEM and SEM techniques [64]. In 2004, the group led by Cakmak synthesized a novel nano-composite composed of MMT NPs and PVC by using the dioctyl phthalate as the plasticizer. Characterizations by
AFM revealed the layered MMT NPs lay preferentially on their basal surfaces. The microstructure analysis implied that if the plasticizer was used in high concentrations, the ability to transfer forces to the MMT NPs through the polymer matrix decreases due to a substantial decrease in viscosity [65].

### 3.2 Characterization techniques

The qualitative determination of NPs in nano-composites can be realized by applying X-ray diffraction (XRD) techniques [66]. In general, it can analyze crystallized materials. XRD is used to determine the identity of crystalline solids based on their atomic structure. Diffraction occurs because X-rays have wavelengths in the order of a few angstroms, which are about the same as the interatomic distances in crystalline solids. Up to now, small angle X-ray scattering (SAXS) and wide X-ray scattering (WAXS) derived from XRD technique have been adopted to analyze the fine structure of the superlattice of the self-assembled NPs. The group lead by Beaucage adopted two-dimensional (2D) SAXS and 2D WAXS to analyze the dispersion and orientations of organically modified MMT used as reinforcement for the HDPE matrix [67]. A researcher from the Air Force Research Laboratory, Ohio, USA, adopted SAXS to examine the structure of organically modified MMT dispersed in toluene and toluene-acetone blends, providing detailed information on the distribution of the stacks and characteristics of the intercalated crystallite [68]. In 2005, the research group lead by Zhu synthesized a semicrystalline polymer/layered silicate nano-composites by blending a solution of a low molecular weight PE oxide (PEO) with an organically modified MMT NPs. The intercalation morphology was studied by temperature-dependent synchrotron wide-angle X-ray diffraction (WAXD). Significant secondary crystallization was observed in the PEO-MMT nano-composites. Reversible deintercalation and intercalation processes were detected during secondary crystallization and subsequent melting of the secondary crystals. On the basis of the two dimensional WAXD results, an interphase layer between the MMT primary particles and PEO lamellar crystals was proposed.

Recently, more and more researches have adopted solid-state nuclear magnetic resonance (SSNMR) to characterize the NP dispersion in a polymer matrix. In 2001, a research group led by Van der Hart from the National Institute of Standards and Technology, Maryland, USA, adopted SSNMR to characterize the MMT in nylon matrix. They applied Fe atoms in the MMT to determine its dispersion in a polymer matrix [69]. Based on these results, they further characterized the phase transition of the MMT NPs and the crystallization process of the MMT within the polymer matrix [70]. EPR is another important analysis technique to characterize the interaction between NPs and a polymer matrix. For instance, researchers from the Max Planck Institute for Polymer Research, Mainz, Germany, used the EPR to directly measure and understand the interface between polymer and organically modified MMT NPs. During the characterization, the phenomenon was observed that some mobility of the MMT increased with the increasing temperature [71].

Surface structure and interaction between surface protecting ligands and atoms can be achieved by absorption spectroscopy, NIR, Fourier transform infrared spectrometry (FTIR) and Raman spectroscopy. In 2003, a research group led by Gleason from Massachusetts Institute of Technology utilized FTIR to study the Si–O stretching peak and its peak shifts. This suggests a method to observe how MMT NPs deform relative to the polymer matrix [72]. In 2004, a research group from the U.S. Department of Energy led by Maupin characterized the MMT-PLA and MMT-PS composites. They used fluorescence dye (Nile Blue A) on imidazolium or ammonium treated MMT, and adopted laser-induced fluorescence spectroscopy as a probe to detect the interaction and exfoliation in nano-composites. As clay exfoliated in PLA, the color of nano-composites changed from purple to red, and spectra from the dye also changed [73].

### 4 Problems and expectations

Not only does the application of nanotechnology in food packaging bring convenience for daily life, it also enhances the development of nanotechnology from basic research to applied areas. However, to understand the migration properties of NPs in composites and toxicity, it is necessary for us to assess fully and effectively the safety of NPs used in food packaging & contact materials. Recently, the U.S. Department of Agriculture and Food and Drug Administration (FDA), and the EU, conducted a brief assessment on NPs and NMs applied in various areas of industry. The relatively scarce scientific data on migration, exposure sources and toxicities indicate the difficulties and problems in properly understanding the nature of NPs. At present, the following problems are identified and need to be resolved to fully assess the safety of NPs in food packaging:

1. More and effective separation and determination techniques are needed to develop and establish the matrix for nano-composites with paper and paperboard, glass and ceramics, and metals;

2. Comprehensive physicochemical characteristics of the applied NPs should be established. These data include size, size distribution, inherent crystal structure, morphologies, surface ligand properties, surface charge, surface reactivity and the aggregation behaviors under different pH conditions;

3. Establish the relationship between particle size, purity and toxicity;

4. As a priority, a methodology needs to be established
for determining and assessing the migration potential and process of NPs from food contact materials to packed food. Compared to the detection techniques for traditional small organic molecular and inorganic compounds, the skills required for the analysis of NPs are more sophisticated. In migration tests, the migratability of NPs in a matrix, dispersion in simulations and alterations in size and morphologies before and after the migration tests bring challenges for successful assessment of the safety of NMs;

(5) Develop an understanding of toxicity following oral intake of a wide range of NMs for which there is likely human exposure;

(6) Generate information on the toxico-kinetic properties of NPs after oral exposure and appropriate dose metrics in relation to the toxicity of NPs. Correlate these data with the physicochemical characteristics to see whether different NPs can be grouped. Generate information on the bioavailability from food and feed for a range of NMs and investigate potential accumulation in different organs and transport through the placenta and into milk. In addition, biotransformation and excretion should be addressed. Develop, improve and validate in silico, in vitro and in vivo test methodologies to assess toxicity of NPs.

The safety assessment of NPs and NMs does not imply restraining the application of NPs and NMs in the food industry. Before it extensively pervades people’s daily life, it is very necessary for us to properly assess the safety of NMs. It is believed that the invention of new and novel detection techniques in the future will give the NMs a reasonable and justified evaluation, which undoubtedly can be considered as a warranty for the public to safely enjoy high technology products in the food industry.

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