



Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

journal homepage: www.elsevier.com/locate/jiec

Nucleic acid interaction with magnetic nanoparticles modified with carbosilane Dendrimers: From water purification to virus detection

Samuel Takvor-Mena^{a, ID}, Andrea Barrios-Gumiel^a, Carlos Rescalvo-Casas^{b, c, ID},
 Felipe Pérez-García^{b, c, d}, Matilde Alique^{e, f, * ID}, Rafael M. Ramírez^{e, f}, Juan Soliveri^b,
 Irene Heredero-Bermejo^{b ID}, Ramón Pérez-Tanoira^{b, c}, Javier Sánchez-Nieves^{a, f, g, * ID},
 Rafael Gómez^{a, f, g}

^a Universidad de Alcalá (UAH), Departamento de Química Orgánica y Química Inorgánica, Research Institute in Chemistry "Andrés M. del Río" (IQAR, UAH), Campus Universitario, 28805 Alcalá de Henares, Madrid, Spain

^b Universidad de Alcalá (UAH), Departamento de Biomedicina y Biotecnología, Campus Universitario, 28805 Alcalá de Henares, Madrid, Spain

^c Hospital Universitario Príncipe de Asturias (HUPA), Departamento de Microbiología Clínica, 28805 Alcalá de Henares, Madrid, Spain

^d Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, 28029 Madrid, Spain

^e Universidad de Alcalá (UAH), Departamento de Biología de Sistemas, Campus Universitario, 28805 Alcalá de Henares, Madrid, Spain

^f Ramón y Cajal Institute for Health Research (IRYCIS), 28034 Madrid, Spain

^g Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Instituto de Salud Carlos III, 28029 Madrid, Spain

ARTICLE INFO

Keywords:

Nucleic acids

Virus detection

Water purification

Magnetic nanoparticles

Carbosilane dendrimers

ABSTRACT

Nucleic acids (NAs) identification is key for diagnosis, but also are contaminants in processes where the highest quality of water is required. In both cases, NAs must be removed from the medium containing them. Magnetic nanoparticles (MNPs) are applied in diagnosis and purification due to the ease of withdrawal by applying an external magnetic field. The covering of MNPs with adequate ligands improves their activity and selectivity. Herein, we report the modification of MNPs with multivalent dendritic carbosilane (CBS) systems, dendrimers and dendrons of different generation, containing a variety of functionalities (carboxylate, ammonium and guanidinium). The effects of dendritic topology, generation, and functional groups on the interaction between the MNPs and NAs were investigated. The results highlight the importance of selecting a combination of these factors to enhance the interaction between the MNPs and the NAs, according to the application. Also, competition tests with a protein, such as bovine serum albumin (BSA), have been carried out to determine the selectivity of the MNPs towards NAs. Finally, an assay in clinical samples to evaluate infection by COVID were done. These findings provide valuable insights for designing MNPs for efficient NAs capture and release with potential applications in diagnosis, NAs delivery or water purification.

Introduction

Nucleic acids (NAs) contain the essential information, such as the genomic information, of any microorganism or cell. This information is unique to each of them, and the identification of a specific NA allows the identification of any organism. In particular, the detection of a variety of infectious diseases caused by pathogenic microorganisms has been achieved through the identification of their unique NAs, as was recently demonstrated during the COVID-19 pandemic [1]. Usually, NAs are extracted from complex biological mixtures, containing small amounts of them, and after treatment, the NAs are replicated using PCR for

identification. Hence, it is primordial to use extractive systems with the highest selectivity for NAs, although these systems must also be able to release the NAs for PCR identification without degradation [2]. A drawback of these systems is the reusability, but the precautions required with the clinical samples make this issue far from reaching.

Mutations during gene replication or damage to chromosomes due to environmental factors can be responsible for genetic disorders that lead to human health problems. Gene therapy, which involves introducing a functional gene copy, is a solution to these diseases [3,4]. The stabilization of this NA is very important for gene therapy, which implies the carrying of NAs to target cells, where NAs modulates gene expression.

* Corresponding authors.

E-mail addresses: matilde.alique@uah.es (M. Alique), javier.sancheznieves@uah.es (J. Sánchez-Nieves).

<https://doi.org/10.1016/j.jiec.2026.01.025>

Received 3 September 2025; Received in revised form 10 December 2025; Accepted 10 January 2026

Available online 12 January 2026

1226-086X/© 2026 The Authors. Published by Elsevier B.V. on behalf of The Korean Society of Industrial and Engineering Chemistry. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

For this, NAs have to be linked to carriers during their route to avoid degradation by RNAase or DNAase enzymes. As part of this mission, carriers must also avoid the displacement of NAs from the carrier/NA complexes by surrounding proteins [5–8]. Although the stability of these complexes is very important, the interaction must be broken once the target is reached, and thus, fine tuning to achieve this goal is mandatory.

On the other hand, residual NAs are related with inflammation processes. Cell-free DNA in blood, exogenous, for example from residual bacterial DNA [9,10] or endogenous, from mitochondria or cell nuclei [11], can act as etiopathogenic agents, causing chronic inflammation in patients with chronic kidney disease (CKD). And as consequence, the risk of developing cardiovascular diseases is high [10]. Traces of DNA cannot be removed from dialysis water and these patients use large amounts of water, 300–800 L/week depending on their prescription [12]. For this reason, improving the elimination of this residual bacterial DNA will bring important health benefits to these patients. Among other requirements, these systems have to be robust enough to avoid their incorporation to the water current, therefore modifications on them must be done through strong interactions. However, recyclability can be here considered as a positive achievement to reduce costs of the process.

For all the applications mentioned above, nanomaterials are very appealing, since their high surface/volume relationship favours interactions with target molecules such as NAs [7]. Magnetic nanoparticles (MNPs) are among the most relevant nanosystems. Together with previous properties, their magnetism clearly upgrades the manipulation of the mixture, since the simple application of an external magnetic field allows the separation of the MNPs from complex mixtures [13–14]. The design of the MNPs must consider the residue/compound to be extracted and the manipulation conditions, avoiding degradation of the MNPs itself. Moreover, recyclability has become a priority. Bare MNPs are common for water purification of metals and other elements, being even included in continuous-flow system where the MNPs were retained with permanent magnets in the tube walls [15]. For this type of system, a controlled change of pH can regenerate the starting material.

However, grafting onto the MNPs surfaces of systems with preferential affinity toward the targets enhances the performance of these nanomaterials [16,17]. In this direction, multivalent ligands such as polymers or dendrimers are the most suitable, since they have an increased affinity when appropriately modified toward NAs than monovalent ligands [18]. These polymers can impart other properties to the new systems, such temperature and pH sensitivity [19], controlling also the aggregation and dispersion of MNPs [16].

Dendritic systems are macromolecules mainly of spherical shape (dendrimers) or conical shape (dendrons) with their surfaces covered with the active groups. They are obtained through controlled step by step synthesis leading to well-defined sizes and shapes, which allows for better establishment of structure/activity relationships. These characteristics make them very attractive for biomedical applications [20]. Particularly, the interaction of NAs with dendritic systems (dendrimers and dendrons) have been deeply explored [21–23].

Carbosilane (CBS) dendrimers and dendrons contain a framework made of C–C and C–Si bonds, which provides high stability and hydrophobicity [24]. Previously, we have published that grafting cationic CBS dendrimers to MNPs was effective at removing bacteria from water dispersions [25] or to extract proteins from cheese whey [26], while grafting carboxylate CBS dendrimers was effective for reducing HIV infection by interacting with the HIV-1 virus [27]. These findings indicated that MNPs supporting CBS dendritic systems were able to interact with microorganisms, due to the presence of the dendritic systems, and that the magnetic core allowed the separation from the media. Since dendritic systems are known to interact with biomacromolecules such as proteins and nucleic acids [24], we decided to study whether MNPs covered with CBS dendritic systems can behave as nucleic acid trapping agents. Additionally, the nanosize of the MNPs is in the order of the size of nucleic acids, a fact that will help to establish better interactions between both systems.

For this, we have modified MNPs (Fe_3O_4) with a library of CBS dendritic systems and explored their interactions with NAs. The effects of topology (dendrimer or dendron) and number (generation G_n) and type of active groups (anionic carboxylate, neutral amine $-\text{NMe}_2$ and $-\text{NH}_2$, and cationic guanidinium (GUA) and ammonium $-\text{NMe}_3^+$) have been evaluated. These modifications should give information about the best combinations, generation, type of groups, or possible modifications, to accomplish with the higher standards for the applications here studied. In addition, studies on NAs release and replication have been performed, to evaluate the applicability of these systems for diagnosis, including final assays with swab samples from COVID patients.

Finally, other point of interest is that the surface of the MNPs here employed, Fe_3O_4 , contains hydroxyl groups, which modification is analogous to other metal oxides materials (ceramics) or cellulose, very common in membranes of filtration/purification systems [28,29]. Also, metal oxides are widely used in various biomedical applications, being grafted to different polymeric materials through these hydroxyl groups [30]. Hence, we can expect that the results here obtained regarding modification/activity of these MNPs with CBS dendrimers are a proof of concept for implementation of related materials within the application fields here described.

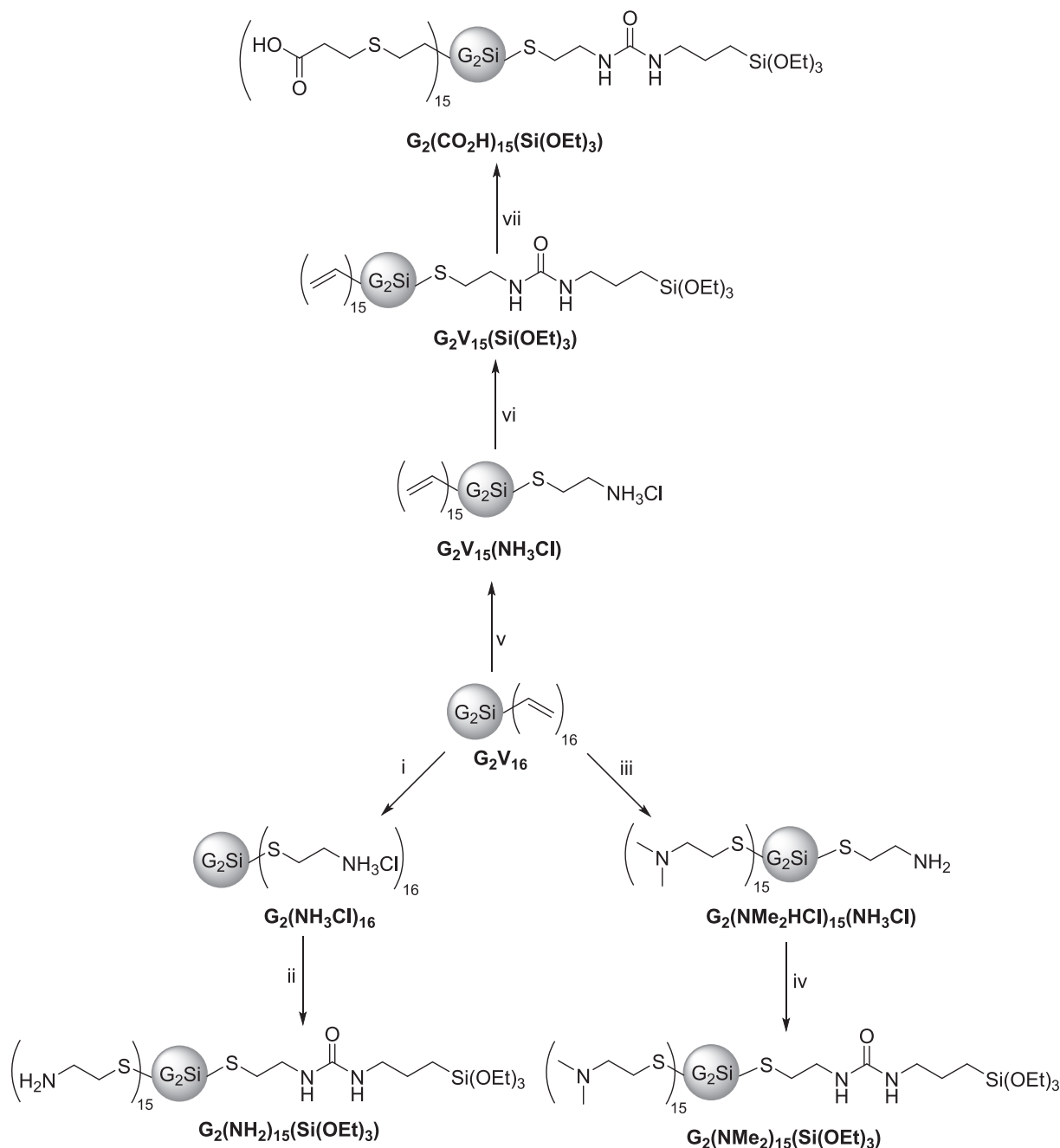
Results and discussion

As commented above, in this work, we have compared the behaviour of different dendritic systems attached to iron oxide (Fe_3O_4) MNPs. We have previously described MNPs covered with cationic ($-\text{NMe}_3^+$) G2 and G3 dendrons ($\text{MNP}@D_2(\text{NMe}_3^+)_4$ and $\text{MNP}@D_3(\text{NMe}_3^+)_8$) and G1 dendrimers ($\text{MNP}@G_1(\text{NMe}_3^+)_7$) (Fig. S1, [25]). To complete the family of MNPs necessary for the assays proposed here, we need to graft a library of G2 CBS dendrimers (with the double of groups than G1 dendrimers) to the MNPs, as the dendrimers modified with $-\text{NMe}_3^+$ groups, but also with neutral amine $-\text{NMe}_2$ and $-\text{NH}_2$, cationic guanidinium (GUA^+) and anionic carboxylate (CO_2^-) functions (Fig. S2). All these systems were built with the same approach, by silanization reactions between heterofunctionalized CBS dendrimers containing one triethoxysilyl moiety and the hydroxyl groups on the MNPs surface. Hence, it is necessary to prepare these heterofunctionalized dendrimers with one silyl moiety and the desired peripheral functions or their precursors. These functions are carboxylic acids ($-\text{CO}_2\text{H}$), and amines $-\text{NMe}_2$ and $-\text{NH}_2$, which were precursors of carboxylate CO_2^- , ammonium $-\text{NMe}_3^+$ and guanidinium $-\text{NHC}(\text{NH}_2)\text{NH}_2^+$ (GUA^+) functions, respectively.

Synthesis of heterofunctional CBS dendrimers

The triethoxysilyl moiety was introduced into the dendrimer by a reaction between a primary amine group $-\text{NH}_2$ of the dendrimer and $(\text{EtO})_3\text{Si}(\text{CH}_2)_3\text{NCO}$, in which the isocyanate fragment reacted selectively with the primary amine group. This primary amine and the second functional groups in the heterofunctional dendrimers were linked using thiol-ene additions, starting from the CBS vinyl dendrimer G_2V_{16} . These two reactions are known by their selectivity, do not generate by-products and the yields are almost quantitative, which made them very useful for dendrimer synthesis. Although the target dendrimers are structurally similar, differing only in the functional groups, the order of introduction of the fragments depended on these groups to avoid side reactions between them and the triethoxysilyl moiety (see experimental part for full description and Scheme 1).

The formation of dendrimer $G_2V_{15}(\text{Si}(\text{OEt})_3)$ was used as a model to confirm the presence of the linker unit by ^1H NMR spectroscopy (Figs. S3–S6). The resonances of this branch are not hidden by the rest of resonances in this dendrimer, situation that occurs when the other vinyl functions are modified by the addition of the other thiols to complete the dendrimer functionalization. The addition of 2-aminoethanethiol to introduce the grafting unit formed a new chain, $\text{SiCH}_2\text{CH}_2\text{S}$, which corresponds with signals around 0.90 and 2.50 ppm (Fig. S3).



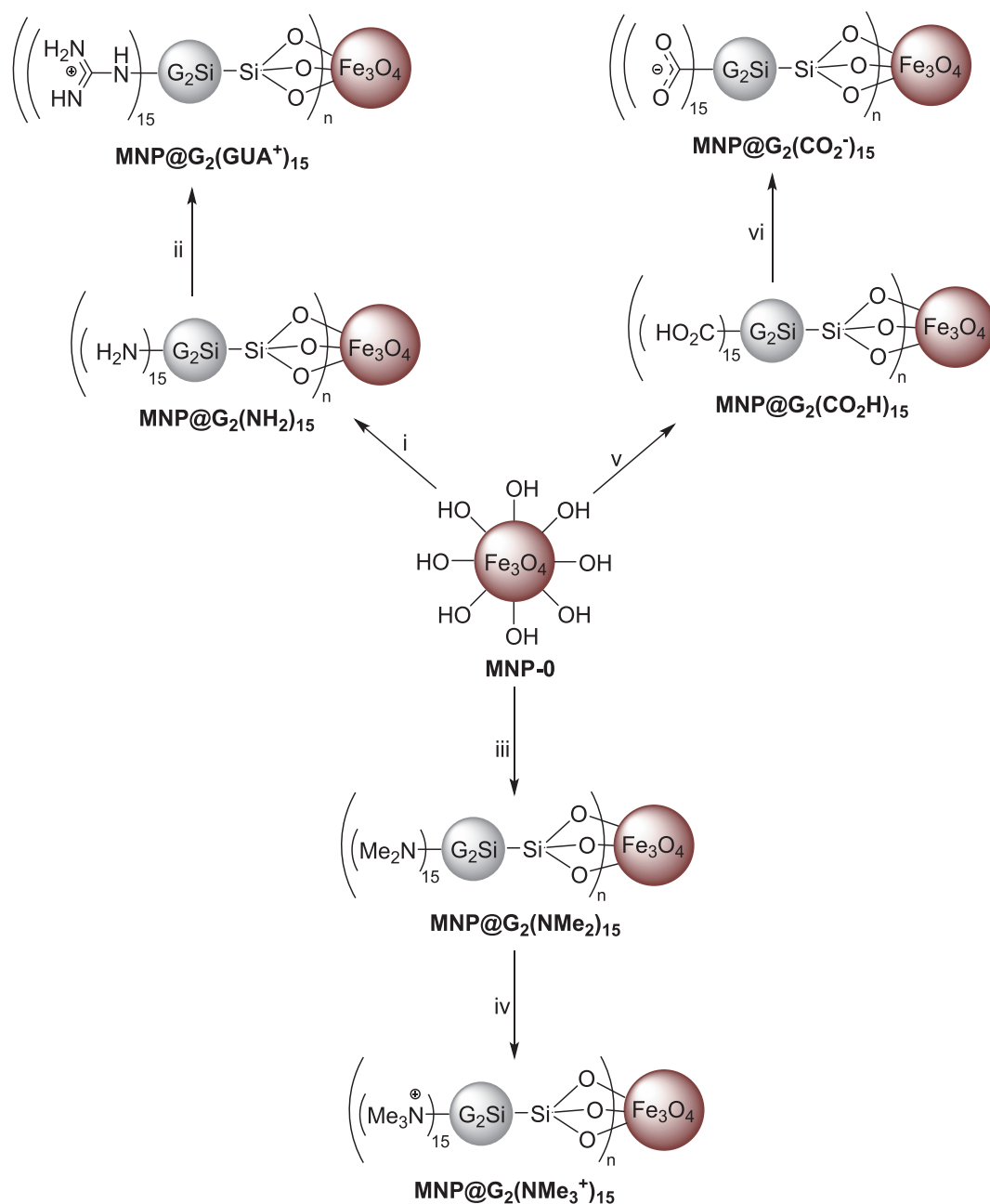
Scheme 1. Synthesis of triethoxysilane dendrimers. i) $HS(CH_2)_2NH_3Cl$, DMPA, hv. $NaHCO_3$; ii) $(EtO)_3Si(CH_2)_3NCO$; iii) $HS(CH_2)_2NH_3Cl$, $HS(CH_2)_2NMe_2HCl$, DMPA, hv. $NaHCO_3$; iv) $(EtO)_3Si(CH_2)_3NCO$; v) $HS(CH_2)_2NH_3Cl$, DMPA, hv. Et_3N ; vi) $(EtO)_3Si(CH_2)_3NCO$; vii) $HS(CH_2)_2CO_2H$, DMPA, hv.

Additionally, resonances for the chain SCH_2CH_2N were also observed at 3.12 (SCH_2) and 3.25 ppm (CH_2NHCO). The second methylene bonded to the urea group appears at 3.35 ppm. The total modification of the dendrimer periphery implied the disappearance of the vinyl resonances (around 5.70 and 6.10 ppm) and the appearance of the resonances of the new functions attached to the dendrimers (triethoxysilyl, amine $-NMe_2$) and of the new chains introduced ($SCH_2CH_2CO_2H$ (Fig. S4) and SCH_2CH_2N (Fig. S5 and S6)), for which the chemical shifts are similar to those described above (see experimental and supporting information). These results are in agreement with related CBS dendrons and dendrimers described for us previously to modify MNPs or silica surfaces [25,27,31].

Synthesis of dendritic MNPs

The Fe_3O_4 core of the MNPs was obtained by the described coprecipitation method [27]. These pristine MNPs obtained in this way (**MNP-0**) were also used in all the assays for comparison with the modified MNPs. As discussed above, the newly modified MNPs, **MNP@ $G_2(CO_2H)_{15}$** , **MNP@ $G_2(NMe_2)_{15}$** , and **MNP@ $G_2(NH_2)_{15}$** , were produced by the reaction between **MNP-0** and excess of the dendrimers modified with the triethoxysilyl units (Scheme 2).

These MNPs were precursors of the corresponding anionic carboxylate MNPs **MNP@ $G_2(CO_2^-)_{15}$** , after basification of **MNP@ $G_2(CO_2H)_{15}$** with $NaHCO_3$, cationic ammonium **MNP@ $G_2(NMe_3^+)_{15}$** , after MeI addition to **MNP@ $G_2(NMe_2)_{15}$** , and cationic guanidium **MNP@ $G_2(GUA^+)_{15}$** , after guanidium exchange between 1H-pyrazole-1-



Scheme 2. Surface functionalization of MNPs. i) $\text{G}_2\text{Si}(\text{NH}_2)_{15}(\text{Si}(\text{OEt})_3)$; ii) 1H-pyrazole-1-carboxamide hydrochloride; iii) $\text{G}_2\text{Si}(\text{NMe}_2)_{15}(\text{Si}(\text{OEt})_3)$; iv) MeI; v) $\text{G}_2\text{Si}(\text{CO}_2\text{H})_{15}(\text{Si}(\text{OEt})_3)$; vi) NaHCO_3 .

carboxamide hydrochloride and $\text{MNP@G}_2(\text{NH}_2)_{15}$, respectively (Scheme 2). All these MNPs were characterized by transmission electron microscopy (TEM), thermal gravimetric analysis (TGA), zeta potential

(ZP) and infrared spectroscopy (FTIR) (data in Table 1).

a) Molar ratio ($\text{Fe}_3\text{O}_4/\text{L}$) obtained by TGA; b) Diameter (nm) obtained by TEM (Figs. S7-S11); c) Number of dendrimers per MNP; d) Number of

Table 1

Data of characterization of MNPs.

	$\text{Fe}_3\text{O}_4/\text{L}^a$	D^b	N L^c	N_{func}^d	$\delta(\text{L})^e$	δ_{Func}^f	ZP^g
MNP-0	—	13.1	—	—	—	—	−23
MNP@D₂(NMe₃⁺)₄	42:1	11.9	224	896	0.59	2.36	+29
MNP@D₃(NMe₃⁺)₈	34:1	11.8	274	2190	0.72	5.76	+30
MNP@G₁(NMe₃⁺)₇	149:1	12.3	83	579	0.18	1.27	+26
MNP@G₂(NMe₂)₁₅	153:1	11.8	76	1140	0.17	2.61	—
MNP@G₂(NH₂)₁₅	141:1	12.0	87	1305	0.19	2.86	—
MNP@G₂(CO₂)₁₅	161:1	12.3	82	1230	0.17	2.59	−37
MNP@G₂(NMe₃⁺)₁₅	155:1	12.1	80	1200	0.17	2.61	+25
MNP@G₂(GUA⁺)₁₅	152:1	11.4	69	1035	0.17	2.54	+42

functions per MNP e) Density of dendrimers (L) per nm^2 on the MNP surface; f) Density of functions per nm^2 (Fun/nm^2) on the MNP surface; g) Zeta potential (mV).

The diameter of all the MNPs was ca. 12 nm, similar to the starting **MNP-0**, as can be expected since TEM analysis only shows the Fe_3O_4 core, meaning this observation that the core does not undergo transformation during the reactions of grafting dendrimers and their modifications (Figs. S7-S11).

The FTIR spectroscopy (Fig. 1) showed the FeO stretching vibration of the magnetite cores, by mean of strong bands at ca. 580 cm^{-1} . The grafting of the CBS dendrimers led to the appearance of bands at about 2900 cm^{-1} , which corresponds with C–H stretching vibrations. For **MNP@G₂(CO₂)₁₅**, the bands about 1580 cm^{-1} and 1450 cm^{-1} confirm the presence of the carboxylate functions, belonging these bands to the asymmetric and symmetric stretching of this function, respectively. In **MNP@G₂(GUA⁺)₁₅**, a broad band centred in 1650 cm^{-1} includes C–N vibrations for the symmetric and antisymmetric stretching and deformation vibrations, and bending vibrations of NH bonds.

TGA analyses revealed that all the MNPs incorporated a similar number of G2 dendrimers after the silanization reaction and hence, they contained similar amounts of functional groups, and more importantly, a similar surface density of functional groups. The number of dendrimers on the MNPs surface was close to that reported previously for MNPs covered with smaller G1 cationic dendrimers, **MNP@G₁(NMe₃⁺)₇** [25], although due to the lesser number of functions in this example, the surface density of groups is ca. the half for **MNP@G₁(NMe₃⁺)₇**.

A remarkable situation was observed when the MNPs covered with G2 dendrimers were compared with MNPs covered with G3 dendrons **MNP@D₃(NMe₃⁺)₈** [25], with fifteen functions per dendrimer vs. eight functions per dendron respectively. In this case, the surface of **MNP@D₃(NMe₃⁺)₈** were clearly more crowded than that of the MNPs modified with G2 dendrimers. This important difference can be attributed to the differences in the length of the anchoring chain. The longer anchoring chain at the focal point of the dendron in **MNP@D₃(NMe₃⁺)₈** would allow for a better dendron accommodation on the surface of the MNPs than the shorter anchoring branch of the heterofunctionalized dendrimers in **MNP@G₂(NMe₃⁺)₁₅**. A similar situation was observed for the MNPs modified with G2 dendrons, **MNP@D₂(NMe₃⁺)₄**, which also

showed higher density of ligands on the MNPs surface, although in this case the number of functions was close to those in the MNPs modified with dendrimers **MNP@G₂(X)₁₅**, (X = CO₂H, NH₂, NMe₂) owing to the higher number of functions in the G2 dendrimers.

The neutral MNPs **MNP@G₂(X)₁₅** were poorly dispersed in water, and their Z potential values could not be obtained. However, after the corresponding modification that led to charged MNPs, they dispersed properly, and the Z potential could be measured. The type of value (positive or negative) was in accordance with the type of functional group, highlighting the clearly more positive Z potential of the guanidinium system **MNP@G₂(GUA⁺)₁₅** than that of the ammonium **MNP@G₂(NMe₃⁺)₁₅**. For the MNPs covered with cationic –NMe₃⁺ functions, there were not a correlation between the density of functions and the Z potential, being all the values similar among them.

DNA capture with MNPs

To evaluate the capture capacity of the different MNPs, an exploratory quantitative assay was performed, using salmon DNA (ca. 2000 base pairs, 50 ppm) and MNPs (500 ppm), measuring the absorbance of the DNA solution before and after treatment with the MNPs (Fig. 2, Table 2). Firstly, we used this test with the family of MNPs modified with cationic –NMe₃⁺ dendritic systems, which gave us hints about the influence of topology (dendron vs. dendrimer) and generation (number of functional groups) on the interaction of the MNPs with DNA (Table 1, entries 1–4).

The results highlighted that the size of the dendritic systems covering MNPs plays the main role, since **MNP@D₃(NMe₃⁺)₈** (entry 2) and **MNP@G₂(NMe₃⁺)₁₅** (entry 4) were the MNPs with higher affinity toward DNA, but the second was covered with half of the functions than the first. This assessment was also supported by the behaviour observed for **MNP@D₂(NMe₃⁺)₄** and **MNP@G₁(NMe₃⁺)₇**. **MNP@D₂(NMe₃⁺)₄** contains ca. 50 % more functions than **MNP@G₁(NMe₃⁺)₇**, but **MNP@D₂(NMe₃⁺)₄** was inactive (entry 1), whereas **MNP@G₁(NMe₃⁺)₇** showed some activity (entry 3). That is, dendrimers spread out of the active moieties from the NPs surface, due to their bigger size, favouring the interaction with DNA. Other authors have demonstrated that increasing the number of amine groups on MNPs favours interaction

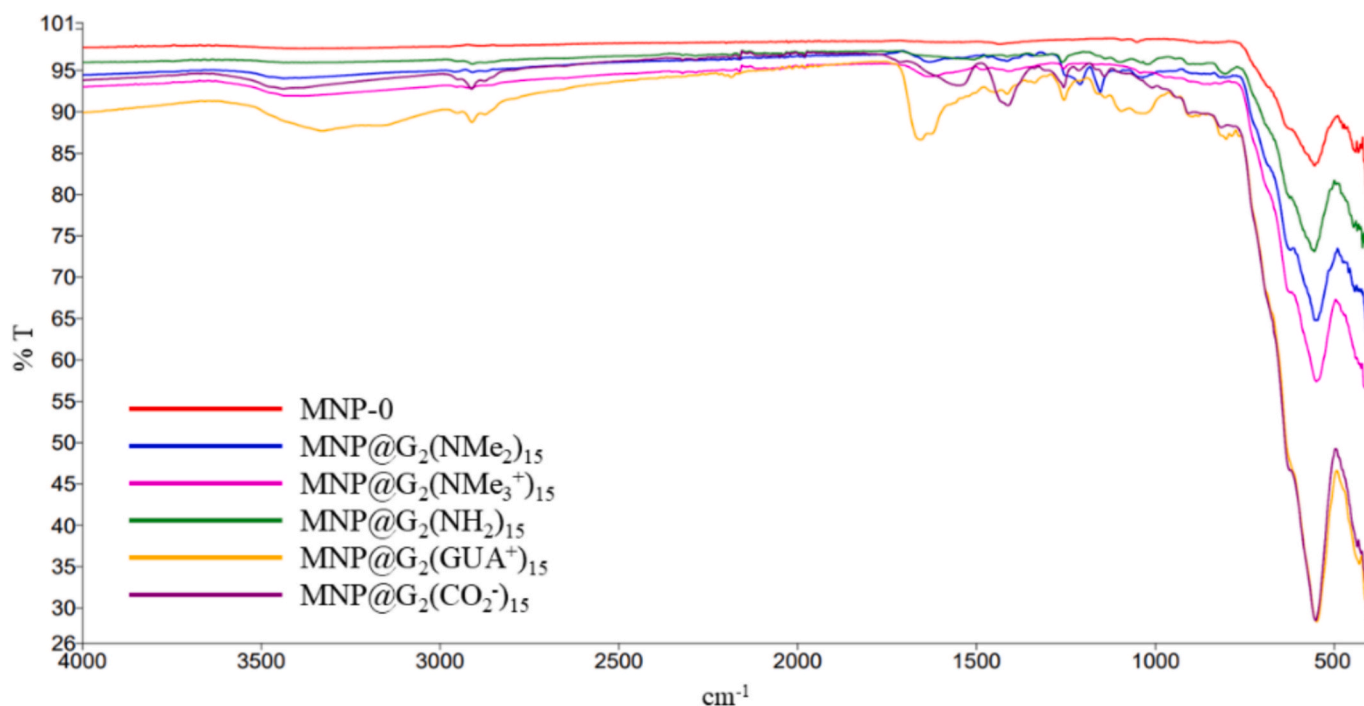


Fig. 1. FTIR spectra of MNPs.

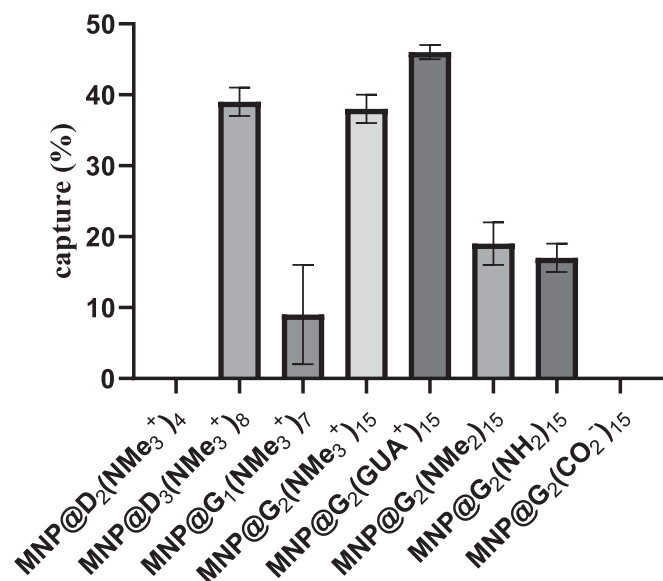


Fig. 2. Graphical representation of DNA capture percentages by different MNPs (from Table 2 (n = 3)). Salmon DNA 50 ppm; MNPs 500 ppm.

Table 2

Capture percentage of salmon DNA (50 ppm) with MNPs (500 ppm) (n = 3).

Entry	MNP	Capture (%)
1	MNP@D ₂ (NMe ₃ ⁺) ₄	0
2	MNP@D ₃ (NMe ₃ ⁺) ₈	39 ± 2
3	MNP@G ₁ (NMe ₃ ⁺) ₇	9 ± 7
4	MNP@G ₂ (NMe ₃ ⁺) ₁₅	38 ± 2
5	MNP@G ₂ (GUA ⁺) ₁₅	46 ± 1
6	MNP@G ₂ (NMe ₂) ₁₅	19 ± 3
7	MNP@G ₂ (NH ₂) ₁₅	17 ± 2
8	MNP@G ₂ (CO ₂) ₁₅	0
9	MNP-0	0

with NAs, and selectivity between DNA and RNA can be modulated depending on amine density [32].

Based on these results, the next step was the comparison of the MNPs covered with G2 dendrimers modified with different functions (anionic carboxylate CO₂⁻, neutral amine -NMe₂ and -NH₂, and cationic -NMe₃⁺ and guanidinium -NHC(NH₂)NH₂⁺ (GUA⁺)) (experiments were done in the same conditions, Fig. 1 and Table 2). All these MNPs contain similar number of active groups.

Of all of them, the most active systems were the cationic MNPs, reaching 38 % and 46 % of DNA retention with MNP@G₂(NMe₃⁺)₁₅ (entry 4) and MNP@G₂(GUA⁺)₁₅ (entry 5), respectively. Neutral MNPs MNP@G₂(NMe₂)₁₅ (entry 6) and MNP@G₂(NH₂)₁₅ (entry 7) captured less amounts of DNA (below 20 %). This result can be justified by pure electrostatic interaction between cationic dendrimers and anionic DNA, being MNP@G₂(GUA⁺)₁₅ the MNPs with more positive Z potential. Finally, for anionic MNP@G₂(CO₂⁻)₁₅ we did not observe capture of DNA at all (entry 8). However, it has been described that polymers and other materials containing carboxylate moieties are able to trap NAs through formation of Na⁺ cations bridges between the carboxylate and the phosphate groups of NAs [33,34], which is the base of commercial NAs extraction systems. This interaction is weak and, probably, in our case there are few carboxylate functions in the medium to capture a measurable quantity of NAs by this technique. With these data of NAs capture, we chose the most active MNPs, MNP@G₂(GUA⁺)₁₅, for subsequent experiments. Moreover, from a synthetic point of view, the G2 CBS dendrimer was built in fewer steps than the G3 CBS dendron.

Once DNA was captured, the possibility of DNA release from the conjugate was analysed. In Table 3 (Fig. 3) we collected the information

Table 3

Capture and release percentage of salmon DNA (starting with 50 ppm of DNA) with MNP@G₂(GUA⁺)₁₅ (500 ppm) (n = 3). The release was done with dextran (Dex)).

Entry	Capture (%)	Release conditions	Release (%)
10	35 ± 6	Dex 1 %, 25 °C	45 ± 6
11	37 ± 2	Dex 1 %, 90 °C	63 ± 5
12	36 ± 2	Dex 2 %, 25 °C	61 ± 5
13	39 ± 2	Dex 2 %, 90 °C	99 ± 3

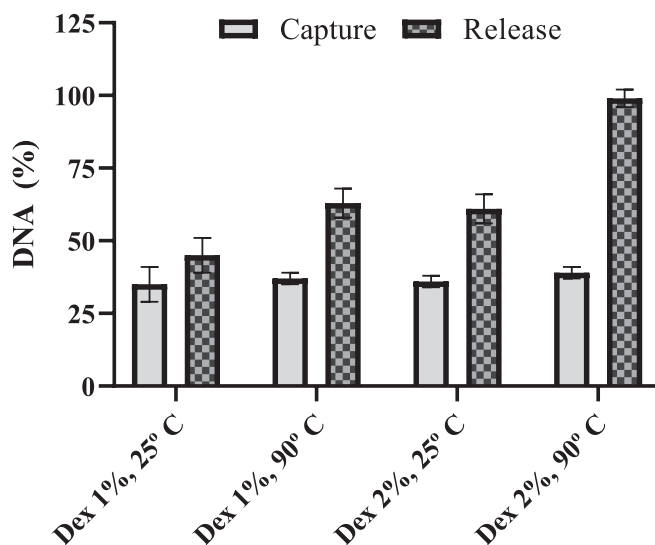


Fig. 3. Effect of dextran (Dex) concentration and temperature on DNA capture and release by MNP@G₂(GUA⁺)₁₅ (from Table 3 (n = 3)). Salmon DNA, 50 ppm; MNP@G₂(GUA⁺)₁₅, 500 ppm.

on this assay using the negative polysaccharide dextran as releasing agent, at 1 % and 2 % and at 25 °C and 90 °C. We managed to release all the DNA trapped with 2 % dextran at 90 °C (entry 13). A similar assay using the surfactant SDS or Tris buffer failed to release any detectable quantity of DNA.

Then, we explored the amount of MNP@G₂(GUA⁺)₁₅ necessary to trap 100 % of the DNA employed (that is, 50 ppm). As can be seen in Table 4 and Fig. 4, we required 2000 ppm of the MNPs for it, being able to release only the 55 % of this DNA (entry 16).

It is also important to determine whether the released DNA can be useful for replication by PCR. With this goal, we performed a similar capture experiment with bacterial DNA from *Escherichia coli* (ca. 1500 bp), due to the relevance of bacterial DNA as an ethiopathogenic agent in inflammatory processes. The capture results with this bacterial DNA were similar to those previously obtained with salmon DNA (Fig. 5), and after treatment for DNA release, we carried out PCR with the supernatant, which was unsuccessful (Fig. 6). This probably means that the extraction procedure required for it degrades the DNA, as demonstrated by a blank experiment treating the DNA with the extraction procedure, in the absence of dextran, which showed the integrity of DNA. However,

Table 4

Percentage of capture and release of salmon DNA (50 ppm) with MNP@G₂(GUA⁺)₁₅ (n = 3) with different amounts of MNPs. The release was done with 2 % of dextran at 95 °C.

Entry	MNP@G ₂ (GUA ⁺) ₁₅	Capture (%)	Release (%)
14	500	35 ± 1	90 ± 3
15	1000	63 ± 1	60 ± 7
16	2000	98 ± 1	55 ± 2
17	4000	99 ± 1	55 ± 5

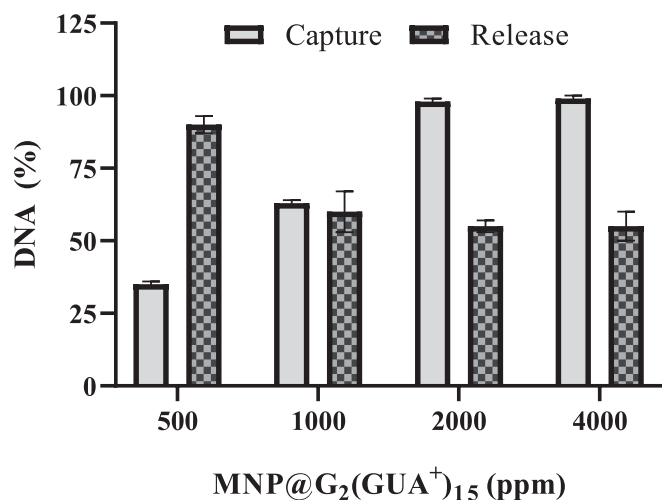


Fig. 4. Effect of MNP@G₂(GUA⁺)₁₅ concentration on DNA capture and release (data from Table 4 (n = 3)). Salmon DNA employed for capture, 50 ppm. The release was done with 2 % dextran solution at 95 °C.

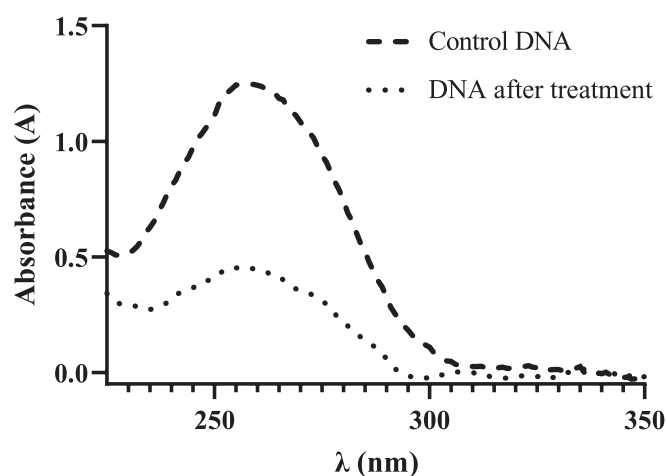


Fig. 5. UV spectra showing the decrease in *Escherichia coli* DNA absorbance after treatment with MNP@G₂(GUA⁺)₁₅ (with 50 ppm of DNA and 1000 ppm of MNPs).

it is known that the presence of dextran in the proportions used in our experiments inhibits the PCR [35].

Analysis of DNA/MNP interaction by DLS and Z potential

The interaction between MNP@G₂(GUA⁺)₁₅ and DNA was analysed by DLS and Z potential measurements. These experiments were performed at a constant concentration of MNPs (500 ppm) by adding 1 ppm of salmon DNA for each measurement (Fig. 7). For the first addition, the DLS showed an important increase in size, from 140 to 333 nm, corresponding with a remarkable reduction of the Z potential from +42 mV to +9.1 mV. Simultaneously, the turbidity of the dispersion increased notably, indicating the formation of aggregates with lower solubility. This phenomenon is produced probably because the net charge is closer to zero, minimizing charge repulsion between MNP/DNA complexes, which favours aggregates formation. Additionally, the positive charge corresponds to the presence of CBS dendrimers on the outer surface of these aggregates, and with low charge, the highly hydrophobic CBS framework reduces notably the dispersibility of the complexes. However, after the second addition, the size of the complex was reduced to ca. 200 nm while the Z potential shifted to negative values (−13.4 mV),

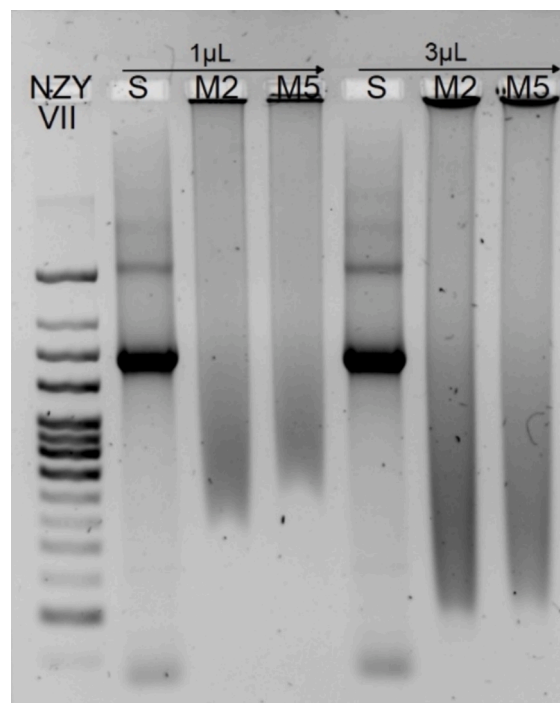


Fig. 6. PCR amplification of DNA from *Escherichia coli*. NZY VII was used as the molecular weight marker. All the assays were done at the same conditions. Untreated control, without MNPs but in the same assay conditions (S) and samples after DNA capture and release treatment (M2 and M5).

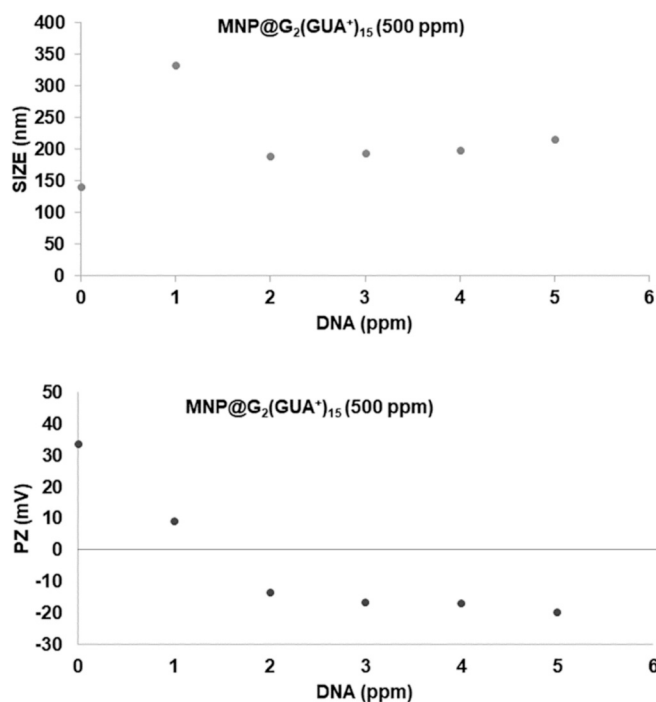


Fig. 7. Variation of size by DLS (top) and Z potential (bottom) of MNP@G₂(GUA⁺)₁₅ with the addition of salmon DNA.

at the same time than turbidity decreased. The addition of more DNA barely modified the size, whereas the Z potential changed slightly to more negative values. It seems that addition of these few amounts of DNA is enough to hide the MNPs surface, favouring the redispersion of the MNP/DNA complexes. However, the fact that this concentration of

MNPs is able to capture DNA up to ca. 17 ppm probably means that DNA rearranges on the MNPs surface to optimize interaction, from a spread situation over MNPs surface (at low DNA concentration) to a partial interaction to accommodate additional DNA molecules, until saturation of the surface is achieved (at high DNA concentration).

Competition DNA/BSA

The presence of proteins in the treatment of biological samples could interfere with the extraction process of NAs, since the abundance of NAs is clearly smaller. In addition, the use of NAs in the field of nanomedicine is of striking relevance for gene therapy treatments. The dendriplex in charge of this transport has to support competition to remove the NAs from the carrier, mainly by proteins such as albumin. For these, we tested the ability of $\text{MNP@G}_2(\text{GUA}^+)_{15}$ to trap DNA in the presence of a model albumin (bovine serum albumin, BSA). According to the results obtained (Table 5), these MNPs were selective toward the trapping of DNA even in the presence of excess BSA. In the assay conditions and due to the structures of these biomacromolecules and the net positive charge of the MNPs, the negative charge of DNA clearly surpass that of BSA, so this result is reasonable. Only huge excess of albumin-like proteins could avoid the interaction of highly positive nanosystems with negative NAs, as has been discussed for the formation of stable complexes with NAs. On the other hand, stronger the complex is, more difficult it is to remove or deliver the NAs from it [21].

Capture of DNA with anionic MNPs

The analysis by UV spectroscopy of the DNA capture with the library of MNPs did not show any capture with the anionic $\text{MNP@G}_2(\text{CO}_2^-)_{15}$. This assay was positive for cationic MNPs, albeit the release procedure of DNA from them degraded the DNA making it unusable for PCR detection. Because of this and that DNA capture by anionic systems is well known, we checked if the anionic $\text{MNP@G}_2(\text{CO}_2^-)_{15}$ could be able to trap traces of DNA for replication by PCR technique, and the results were compared with the commercial magnetic extraction kit Magnetic Bead Viral 200C (Seegene). As for $\text{MNP@G}_2(\text{GUA}^+)_{15}$, we carried out the experiment with bacterial DNA from *E. coli*, following the experimental conditions described in Table 2, with an ionic strength of 1 M NaCl. In this case, the PCR assay gave positive result (Fig. 8), meaning that the anionic $\text{MNP@G}_2(\text{CO}_2^-)_{15}$ captured traces of DNA through clearly weaker interaction than the cationic MNPs. The result obtained was similar than using the commercial system.

Use of anionic $\text{MNP@G}_2(\text{CO}_2^-)_{15}$ for identification of COVID in real samples

Since $\text{MNP@G}_2(\text{CO}_2^-)_{15}$ were able to capture DNA for PCR replication, we tested these MNPs with real samples of patients COVID positive to evaluate these MNPs for possible real applications. These samples had been previously analysed and classified according to their threshold cycle (CT), below 30 or over 30 (control value, MagCore® system). The results were also compared with the commercial system Magnetic Bead Viral 200C (Seegene), in both cases using the same protocol treatment. For this assay, we selected five samples with a CT less than 30 and four

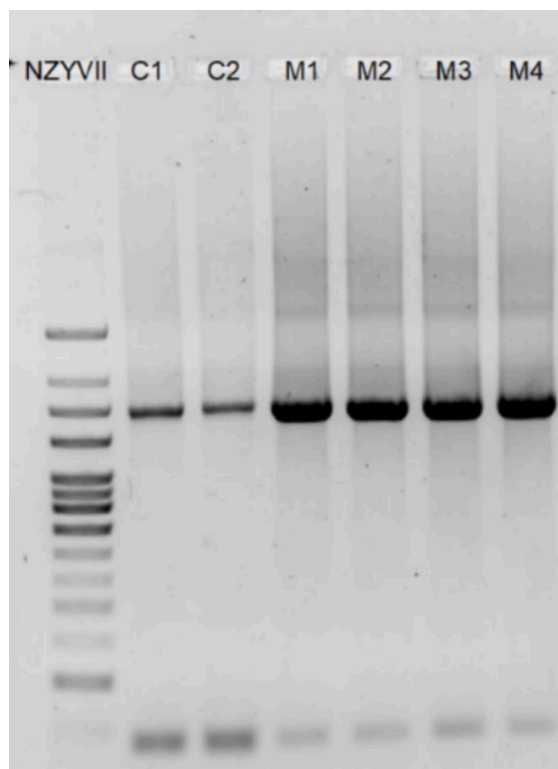


Fig. 8. PCR amplification of DNA from *Escherichia coli*. NZY VII was used as the molecular weight marker. C1 and C2 correspond to untreated controls, while M1 and M2 are samples treated with commercial Magnetic Bead Viral 200C (Seegene), and M3 and M4 with $\text{MNP@G}_2(\text{CO}_2^-)_{15}$.

samples with a CT greater than 30, for a total of 30 replicates. The results obtained are shown in Tables S1-S11.

The results obtained from comparing the use of commercial and modified MNPs with anionic dendritic systems indicate that both exhibit low efficiency at low viral loads. However, under the conditions of high viral load, both systems can adsorb the viral NA, yielding acceptable CT values, being only slightly higher than those of the control. That is, the sensitivity, dividing the number of positive data by the number of total experiments, of the anionic MNPs $\text{MNP@G}_2(\text{CO}_2^-)_{15}$ and commercial systems for the samples with CT below 30 was 100 % (Tables S1 and S3-S7, supernatant B), whereas for samples with CT above 30, the sensitivity were for both systems rather low (25 % for commercial and 19 % for $\text{MNP@G}_2(\text{CO}_2^-)_{15}$, Tables S2 and S8-S11, supernatant B).

Conclusions

The ability of MNPs grafted with CBS dendritic systems to trap NAs depends on several factors as size, topology and charge of dendritic systems. In general, the higher the generation, the higher capture ability. This is mainly because bigger systems offer better their active moieties for interaction with the NAs, that is, they are more distant from the MNPs surface. Regarding the charge, clearly the electrostatic interaction between anionic DNA and cationic MNPs favours this interaction, in contrast to Na^+ ions bridging anionic DNA and anionic MNPs.

According to these results, MNPs grafted with cationic systems can be proposed as purification systems, whereas those grafted with anionic systems confirm their applicability in detection tests through PCR processes. It is important to note that these MNPs are stable in a wide range of temperature and pHs, among other facts due to the presence of the CBS dendrimers. However, in the case of cationic systems, due to the strong interaction of NAs with the MNPs, they lack recyclability, a goal for water purification processes.

Table 5

Percentage of DNA salmon captured with $\text{MNP@G}_2(\text{GUA}^+)_{15}$ in the presence of BSA (DNA, 50 ppm; $\text{MNP@G}_2(\text{GUA}^+)_{15}$, 2000 ppm; n = 2).

Entry	BSA	Capture (%)
18	0	98 ± 1
19	250	98 ± 1
20	500	99 ± 1
21	1000	99 ± 1
22	2000	99 ± 1

However, if the sensibility of these materials needs improvement or for applications as gene carriers, a combination of cationic surface, for strong interaction, with modulators of the interaction, to ease the release of the NAs, is primordial. In this regard, grafting MNPs with cationic moieties containing degradable bonds could be a solution. Other drawback of them is the inherent higher difficulty of the synthesis of dendrimers in comparison with related polymers, which could hamper scalability of these MNPs. To solve this, hyperbranched polymers, which combine structures similar to dendrimers but produced more easily by one-pot synthesis, are an important alternative.

Experimental section

The synthesis of dendrimers and MNPs was carried out under inert conditions using dry solvents. Thiol-ene reactions were carried out with a novaLIGHT TQ150-Z0 lamp from Peschl Ultraviolet with maximum energy at 365 nm in normal glassware after deoxygenation bubbling argon. The NMR spectra were recorded on Bruker Avance Neo 400 at ambient temperature (400.13 MHz for ^1H), 100.60 MHz for ^{13}C). Chemical shifts (δ) are given in ppm. ^1H and ^{13}C resonances were measured relative to solvent peaks considering TMS = 0 ppm. IR spectra were recorded in a IR-FT Perkin Elmer Frontier and elemental analyses were carried out on a PerkinElmer 240C instrument. Reagents and solvents were obtained from commercial sources, and compounds **G₂SiV₁₆** [36], **G₂SiV₁₅(NH₃Cl)** [37], **G₂Si(NMe₂HCl)₁₅(NH₃Cl)** [37], **G₂Si(NH₃Cl)₁₆** [38], **MNP-0** [27], **MNP@D₂(NMe₃⁺)₄** [25], **MNP@D₃(NMe₃⁺)₈** [25], and **MNP@G₁(NMe₃⁺)₇** [25] were produced according to literature. TEM images were obtained with a ZEISS EM TEM equipment with 30 μm lenses and 1 K CCD Camera on the lateral was used. TGA were obtained in a TGA 550 from TA Instruments. Z-Potential and DLS were measured in a Zetasizer Nano ZS.

Synthesis of CBS dendrimer **G₂Si(CO₂H)₁₅(Si(OEt)₃)**.

G₂SiV₁₅(Si(OEt)₃). Compound **G₂SiV₁₅(NH₃Cl)** (0.91 g, 0.57 mmol) was dissolved in hexane, Et₃N (0.24 mL, 1.71 mmol) was added and the solution was stirred for 30 min. Afterward, (EtO)₃Si(CH₂)₃NCO (0.14 mL, 0.57 mmol) was added and the solution was stirred overnight. Afterward, solution was filtered and solvent was removed under vacuum, obtaining **G₂SiV₁₅(Si(OEt)₃)** as a colourless oil. This compound was used without further treatment for next reaction, considering quantitative yield.

^1H NMR (CDCl₃): δ (ppm) = -0.09 (s, 12H, SiCH₃); 0.13 (s, 24H, SiCH₃); 0.53 (m, 32H, SiCH₂CH₂CH₂Si; SiCH₂CH₂CH₂Si); 0.63 (m, 2 H, CH₂Si(OCH₂CH₃)₃); 0.70 (m, 16H, SiCH₂CH₂CH₂Si); 0.92 (m, 2 H, SiCH₂CH₂Si); 1.22 (m, 9 H, Si(OCH₂CH₃)₃); 1.35 (m, 24H, SiCH₂CH₂CH₂Si); 1.79 (m, 2 H, NHCH₂CH₂CH₂Si); 2.54 (m, 2 H, SiCH₂CH₂Si); 2.66 (m, 2 H, SCH₂CH₂NH); 3.15 (m, 2 H, NHCH₂CH₂CH₂Si); 3.36 (m, 2 H, SCH₂CH₂NH); 3.82 (m, 6 H, Si(OCH₂CH₃)₃); 5.69 (m, 15 H, SiCHCH₂); 6.06 (m, 30 H, SiCHCH₂). ^{13}C NMR (CDCl₃): δ (ppm) = -5.1, -5.0 (SiCH₃); 9.3 (NHCH₂CH₂CH₂Si); 14.6 (SiCH₂CH₂Si); 17.3 (Si(OCH₂CH₃)₃); 18.4–20.0 (SiCH₂CH₂CH₂Si); 22.9 (NHCH₂CH₂CH₂Si); 26.3 (SiCH₂CH₂Si); 32.6 (SCH₂CH₂NH); 39.7 (NHCH₂CH₂CH₂Si); 42.7 (SCH₂CH₂NH); 68.5 (Si(OCH₂CH₃)₃); 132.9 (SiCHCH₂); 138.0 (SiCHCH₂); 158.7 (C=O).

G₂Si(CO₂H)₁₅(Si(OEt)₃). A solution of HS(CH₂)₂CO₂H (0.75 mL, 8.55 mmol, 15 eq) and DMPA (2,2-dimethoxy-2-phenylacetophenone, 22.2 mg, 1 % mol with respect to vinyl groups) was added to a solution of **G₂SiV₁₅(Si(OEt)₃)** (1.03 g, 0.57 mmol, 1 eq) in THF. The mixture was bubbled with Argon and stirred under ultraviolet light for 4 h. Afterward, volatiles were removed under vacuum and compound **G₂Si(CO₂H)₁₅(Si(OEt)₃)** was obtained as a yellowish oil. This compound was used without further treatment for modification of MNPs, considering quantitative yield.

^1H NMR ((CD₃)₂CO): δ (ppm) = 0.01 (s, 12 H, SiCH₃); 0.10 (s, 24H, SiCH₃); 0.67 (m, 32 H, SiCH₂CH₂CH₂Si, SiCH₂CH₂CH₂Si); 0.74 (m, 18 H, SiCH₂CH₂CH₂Si, CH₂Si(OCH₂CH₃)₃); 0.97 (m, 32 H, SiCH₂CH₂Si); 1.12 (m, 9 H, Si(OCH₂CH₃)₃); 1.45 (m, 24H, SiCH₂CH₂CH₂Si); 1.76 (m,

2 H, NHCH₂CH₂CH₂Si); 2.62–2.79 (m, 94 H, SiCH₂CH₂S, SCH₂CH₂COOH, SCH₂CH₂COOH, SCH₂CH₂NH); 3.09 (m, 2 H, NHCH₂CH₂CH₂Si); 3.31 (m, 2 H, SCH₂CH₂NH); 3.57 (m, 6 H, Si(OCH₂CH₃)₃). ^{13}C NMR ((CD₃)₂CO): δ (ppm) = -4.90, -4.70 (SiCH₃); 9.5 (NHCH₂CH₂CH₂Si); 15.1 (SiCH₂CH₂Si); 17.4 (Si(OCH₂CH₃)₃); 18.3–20.8 (SiCH₂CH₂CH₂Si, NHCH₂CH₂CH₂Si); 26.8 (SiCH₂CH₂Si); 28.4 (SCH₂CH₂COOH); 29.3 (SCH₂CH₂NH); 36.1 (NHCH₂CH₂CH₂Si); 39.4 (SCH₂CH₂NH); 58.4 (SCH₂CH₂COOH); 68.7 (Si(OCH₂CH₃)₃); 160.4 (C=O); 177.2 (COOH). Elemental analysis for C₁₃₇H₂₅₉N₂O₃₄Si₁₆Si₁₄ (3384.72 g mol⁻¹): calc.: C 48.62, H 7.71, N 0.83; obt.: C, 50.66; H, 7.92; N 1.10. This data belongs to the crude product of the reaction, since no purification was done in this step.

Synthesis of carbosilane dendrimer **G₂Si(NMe₂)₁₅(Si(OEt)₃)**.

Compound **G₂Si(NMe₂HCl)₁₅(NH₃Cl)** (2.01 g, 0.54 mmol, 1 eq) was solved in DMF, Et₃N (2.40 mL, 17.3 mmol) was added and the solution was stirred for 30 min. Then, (EtO)₃Si(CH₂)₃NCO (0.13 mL, 0.54 mmol, 1 eq) was added and the solution was stirred overnight. Afterward, the solution was filtered, and the volatiles were removed under vacuum, yielding **G₂Si(NMe₂)₁₅(Si(OEt)₃)** as a yellowish oil. This compound was used without further treatment for modification of MNPs, considering quantitative yield.

^1H NMR (CD₃Cl): δ (ppm) = -0.11 (s, 12 H, SiCH₃); -0.02 (s, 24 H, SiCH₃); 0.50 (m, 32 H, SiCH₂CH₂CH₂Si, SiCH₂CH₂CH₂Si); 0.58 (m, 18 H, SiCH₂CH₂CH₂Si, CH₂Si(OCH₂CH₃)₃); 0.86 (m, 32 H, SiCH₂CH₂Si); 1.20 (m, 9 H, Si(OCH₂CH₃)₃); 1.26 (m, 24 H, SiCH₂CH₂CH₂Si); 1.57 (m, 2 H, NHCH₂CH₂CH₂Si); 2.21 (s, 90 H, NMe₂); 2.45–2.79 (m, 94 H, SiCH₂CH₂S, SCH₂CH₂NMe₂, SCH₂CH₂NMe₂, SCH₂CH₂NH); 3.15 (m, 2 H, NHCH₂CH₂CH₂Si); 3.32 (m, 2 H, SCH₂CH₂NH); 3.77 (m, 6 H, Si(OCH₂CH₃)₃). ^{13}C NMR (CDCl₃): δ (ppm) = -5.10, -4.90 (SiCH₃); 9.1 (NHCH₂CH₂CH₂Si); 14.8 (SiCH₂CH₂Si); 17.4 (Si(OCH₂CH₃)₃); 18.0–19.8 (SiCH₂CH₂CH₂Si); 23.4 (NHCH₂CH₂CH₂Si); 27.9 (SiCH₂CH₂Si); 30.0 (SCH₂CH₂NMe₂); 32.8 (SCH₂CH₂NH); 39.5 (NHCH₂CH₂CH₂Si); 43.1 (SCH₂CH₂NH); 45.3 (NMe₂); 59.4 (SCH₂CH₂NMe₂); 68.3 (Si(OCH₂CH₃)₃); 158.3 (C=O). Elemental analysis for C₁₅₂H₃₄₉N₁₇O₄₅Si₁₆Si₁₄ (3385.72 g mol⁻¹): calc.: C 53.92, H 10.39, N 7.03; obt.: C, 56.39; H, 9.52; N 9.64. This data belongs to the crude product of the reaction.

Synthesis of carbosilane dendrimer **G₂Si(NH₂)₁₅(Si(OEt)₃)**.

Compound **G₂Si(NH₃Cl)₁₆** (1.82 g, 0.55 mmol, 1 eq) was dissolved in EtOH, Et₃N (0.76 mL, 5.50 mmol, 10 eq) was added and the solution was stirred for 30 min. Afterward, (EtO)₃Si(CH₂)₃NCO (0.14 mL, 0.55 mmol, 1 eq) was added and the solution was stirred overnight. Next, an aliquot was taken from the solution and the volatiles were removed under vacuum to check the reaction by NMR spectroscopy, which confirmed the formation of the urea group. If the volatiles are totally removed, usually this compound aggregates becoming impossible to solve it again. This compound was used without further treatment for modification of MNPs, considering quantitative yield.

^1H NMR (CD₃Cl): δ (ppm) = -0.11 (s, 12 H, SiCH₃); -0.02 (s, 24H, SiCH₃); 0.50 (m, 32 H, SiCH₂CH₂CH₂Si; SiCH₂CH₂CH₂Si); 0.58 (m, 18 H, SiCH₂CH₂CH₂Si; CH₂Si(OCH₂CH₃)₃); 0.86 (m, 32 H, SiCH₂CH₂Si); 1.18 (t, 9 H, Si(OCH₂CH₃)₃); 1.26 (m, 24 H, SiCH₂CH₂CH₂Si; SiCH₂CH₂CH₂Si); 2.45 (m, 32 H, SCH₂CH₂N); 2.52 (m, 32 H, SiCH₂CH₂S); 2.59 (m, 32 H, SCH₂CH₂N); 3.12 (m, 2 H, SCH₂CH₂NH); 3.25 (m, 2 H, SCH₂CH₂NH); 3.32 (m, 2 H, NHCH₂CH₂CH₂Si); 3.77 (m, 6 H, Si(OCH₂CH₃)₃). Elemental analysis was not done due to the impossibility of isolation without aggregation.

Surface functionalization of the MNPs.

For MNPs yield calculation, the limiting reactant is the starting MNPs used to graft dendrimers or to post-functionalize (for example **MNPs@G₂(NH₂)₁₅** for synthesis of **MNPs@G₂(GUA-Cl)₁₅**). The theoretical mass increment that will suffer the new MNPs after functionalization is obtained from TGA data.

MNPs@G₂(CO₂H)₁₅. An ethanol solution of **MNP-0** (150 mL, 0.131 g, 0.57 mmol) was sonicated for 15 min. After that a dendrimer ethanol solution (50 mL) of **G₂Si(CO₂H)₁₅(Si(OEt)₃)** (1.93 g, 0.57 mmol) was

added, the solution was bubbled with argon and sonicated for 15 min more. Then, the mixture was stirred overnight at room temperature. The product was washed with EtOH (3 x 30 mL) and with acetone (3 x 30 mL) and finally dried under vacuum. Yield: 0.115 g, 80 %.

MNPs@G₂(CO₂Na)₁₅: The carboxylate MNP was prepared by the addition of NaHCO₃ (ratio 20:1 NaHCO₃:carboxyl group, 0.50 mmol, 0.084 g) to a dispersion of MNPs@G₂(CO₂H)₁₅ (0.115 g) in H₂O/MeOH (1:1). The mixture was stirred for 2 h at room temperature. Then, the product was washed three times with distilled water, three with EtOH and three times with acetone and finally dried under vacuum. Yield: 0.095 g, 83 %.

MNPs@G₂(NMe₂)₁₅: A DMF solution of MNP-0 (150 mL, 0.125 g, 0.54 mmol) was sonicated for 15 min. After that, a dendrimer DMF solution (50 mL) of G₂Si(NMe₂)₁₅(Si(OEt)₃) (0.54 mmol, 1.83 g) was added, the solution was bubbled with Argon and sonicated for 15 min more. Then, the mixture was stirred overnight at room temperature. The product was washed three times with EtOH and three times with acetone and finally dried under vacuum. Yield: 0.107 g, 78 %.

MNPs@G₂(NMe₃I)₁₅: A DMF solution of MNPs@G₂(NMe₂)₁₅ (0.107 g) was sonicated for 15 min. MeI (ratio 20:1 MeI:NMe₂ group, 0.94 mmol, 0.060 µL) was added to the mixture and the solution was stirred and heated at 60 °C for 48 h. The product was washed three times with acetone and finally dried under vacuum. The product was washed three times with EtOH and three times with acetone and finally dried under vacuum. Yield: 0.086 g, 81 %.

MNPs@G₂(NH₂)₁₅: An ethanol solution of MNP-0 (150 mL, 0.127 g, 0.55 mmol) was sonicated for 15 min. After that a dendrimer ethanol solution (50 mL) of G₂Si(NH₂)₁₅(Si(OEt)₃) (1.63 g, 0.55 mmol) was added, the solution was bubbled with argon and sonicated for 15 min more. Then, the mixture was stirred overnight at room temperature. The product was washed three times with ethanol and dried under vacuum. The product was washed three times with ethanol, three times with acetone and dried under vacuum. Yield 0.116 g, 83 %.

MNPs@G₂(GUA-Cl)₁₅: An ethanol solution of MNPs@G₂(NH₂)₁₅ (0.100 g) was sonicated for 15 min. 1H-pyrazole-1-carboxamidinium hydrochloride (0.053 g, 0.36 mmol, 10-fold excess) and Et₃N (1 mL, excess) was added to the mixture and the solution was stirred and heated at 60 °C for 3 days. The product was washed three times with ethanol, three times with acetone and dried under vacuum. Yield: 0.080 mg, 81 %.

DNA extraction and PCR amplification

DNA was extracted from an *Escherichia coli* fresh culture (24 h at 37 °C with slightly agitation) using a commercial kit and following the manufacturer's instructions (NZYMicrobial gDNA Isolation Kit, NZY Tech). Then, the PCR was performed mixing Taq, primers (27F: GAGTTTGATCMTGGCTCAG, 1492R TACGGYTACCTTGTTACGACTT) and DNA template. Cycling conditions established were: 94 °C for 2 min, followed by 35 cycles of 94 °C for 15 s, 60 °C for 15 s and 68 °C for 20 s (Mastercycler personal. Eppendorf). The PCR products were purified using a commercial kit (GeneJET PCR Purification Kit, ThermoScientific).

To confirm the amplicon size (1500 bp), an agarose gel was run and, finally, the DNA concentration was quantified (EpochTM, BioTek Instruments).

DNA capture and release experiments.

MNPs were dispersed in 50 µL Milli-Q water (2-fold the final concentration required, see Tables 2-4) and sonicated for 10 min. The resulting suspension was added to a salmon DNA solution (100 ppm in 50 µL), and the mixture was subjected to an orbital shaker for 10 min. The final volume was adjusted to 100 µL in 0.2 mL PCR tubes, corresponding to MNPs and DNA concentrations according to experimental requirements (see Tables 2-4). The mixture was then subjected to magnetic separation for 15 min and the supernatant was analyzed using a Nanodrop spectrophotometer (n = 3). As a control, a DNA solution without MNPs was prepared at the same concentration and volume, and directly measured with the Nanodrop to determine the initial DNA

content available for capture.

For the release experiments, a dextran solution in Milli-Q water (100 µL, 1 % or 2 % dextran, see Tables 3 and 4, Figs. 2 and 3) was added to the MNP-DNA complexes obtained after magnetic separation, following above procedure, and incubated for 10 min (at 25 °C or 95 °C). The suspension was then magnetically separated for 15 min, and the supernatant was analysed again using a Nanodrop spectrophotometer (n = 3). In the case of anionic MNP@G₂(CO₂)₁₅, the dispersions were prepared in 2 M NaCl Milli-Q water, and pure Milli-Q water was used for the release step instead of dextran (n = 3).

Competition assay DNA/BSA with MNP@G₂(GUA⁺)₁₅.

A mixture of salmon DNA (100 ppm in 50 µL) and BSA (100, 200, 500, 1000, 2000 and 4000 ppm in 50 µL) were dispersed in MilliQ water. Then, the mixture was added to a previously sonicated MNPs solution (4000 ppm in 50 µL), the final volume was adjusted to 100 µL in 0.2 mL PCR tubes and the mixture was subjected to an orbital shaker for 10 min. With this procedure, the final concentration of each biomacromolecule and MNPs is half the initial concentration. The mixture was then subjected to magnetic separation for 15 min and the supernatant was analyzed using a spectrophotometer Denovix DS-11 (n = 3).

Analysis of DNA/MNP interaction by DLS and Z potential.

A dispersion of MNPs in 1 mL of MilliQ water at a concentration of 500 ppm was sonicated for 10 min. Then, a DNA solution in MilliQ water at a concentration of 1000 ppm was prepared, and 1 µL aliquots were successively added to the MNPs suspension prior to each measurement, resulting in an incremental increase of approximately 1 ppm DNA per addition. After each addition, the sample was gently mixed and allowed to equilibrate for 5 min before measuring the hydrodynamic diameter by DLS and the zeta potential in a Malvern Zetasizer (nano series).

Use of anionic MNP@G₂(CO₂)₁₅ for identification of COVID in real samples.

For the control CT values, we used a MagCore® of RBC Bioscience Corp and followed the automatic procedure described for this device by the manufacturer, using Magnetic Bead Viral 200C (Seegene). On the other hand, the CT values obtained for the comparison of Commercial magnetic beads (Magnetic Bead Viral 200C (Seegene)) and our MNPs MNP@G₂(CO₂)₁₅ were obtained following the procedure described below.

1. Dispersion of MNPs. MNPs were dispersed by adding 4.0 mg of nanoparticles to 2 mL of saline solution to obtain a final concentration of 2.0 mg/mL. The suspension was sonicated for 10 min to ensure homogenous dispersion.
2. Sample control. For control samples, 200 µL of clinical sample was mixed with 200 µL of AVL viral lysis buffer prior to automated RNA extraction and RT-PCR analysis.
3. Treatment of samples with MNPs. First, 100 µL of patient sample and 100 µL of NPMs were added to a PCR eppendorf. The mixture was subjected to an orbital shaker for 30 min. This allows the virus to interact with the NPMs. After this period, the tubes were placed in the PCR eppendorf magnet for 5 min to retain the nanoparticles at the bottom and removed 200 µL of supernatant in 250 µL eppendorfs that will be treated by automated RNA extraction and PCR (Supernatant A).

Following removal of Supernatant A, 200 µL of elution buffer was added directly to the MNPs retained in the tube. Then, vortexed for a few seconds and afterwards, the eppendorfs were kept rotating in the orbital tube shaker. At the end of the time, they were placed in the eppendorf PCR magnet for 5 min.

The supernatant was added to another PCR eppendorf with 200 µL of lysis buffer and 20 µL of 20 µl of the mixture of 10 µl of Carrier RNA (1 mg/mL) and 20 µl of proteinase K (10 mg/mL) for subsequent RNA extraction by MagCore HF16 RBC Bioscience, Taipei, Taiwan) and subsequent amplification and detection of SARS-CoV-2 by VIASURE (Certest Biotech, Zaragoza, Spain).

RNA extraction from nanoparticles. The RNA extraction was performed using the MagCore HF16 using the method listed below

- Add 200 μ L of sample into MagCore sample tubes.
- Add 200 μ L of AVL viral lysis buffer over the sample to inactivate it.
- Add 20 μ L of the mixture of 10 μ L of Carrier RNA (1 mg/ml) and 20 μ L of proteinase K (10 mg/ml).
- Pipette 200 μ L of the mixture into a sample tube.
- Place the sample tubes in W1 of the T-Rack.
- Place the elution tube in W4 of the T-Rack.
- Place the pipette tip in W3 of the T-Rack.
- Run Code 203 (HF16, Compact, Plus II) 203B (Super, HF16 Plus) in MagCore® to avoid adding internal control.

SARS-CoV-2 RNA detection by RT-PCR

Extracted RNA was analyzed using the VIASURE SARS-CoV-2 Real Time PCR Detection Kit (Certest Biotech, Zaragoza, Spain). For amplification, 15 μ L of rehydration buffer was dispensed into each well of the PCR plate, followed by 5 μ L of extracted RNA (sample, positive control, or negative control). PCR amplification was performed using the following thermal cycling conditions: 1) 45 °C for 15 min; 2) 95 °C for 2 min; 3) 95 °C for 10 s; 4) 60 °C for 50 s; 5) Repeat steps 3–4 for 44 cycles. Detection was conducted on a compatible real-time PCR platform according to manufacturer instructions.

Sensitivity. This value was obtained dividing the number of positive data by the number of total experiments. These data were obtained for supernatants B, which are the ones that retain the SARS-CoV-2 RNA after virus degradation and treatment for RNA extraction (obtained from Tables S1–S11).

Supplementary material.

NMR spectra, TEM images are collected in the supplementary material.

Funding.

This research has been funded by grants from the Instituto de Salud Carlos III (ISCIII) and co-funded by Fondos Europeo de Desarrollo Regional (FEDER) (PI19/00240), grants EPU-INV2020014 from Comunidad de Madrid and UAH, grants 2020/00375/001, 2021/00059/001, 2024/00300/001-GP2024-02 and EPU-INV-UAH/2022/001 form UAH. This research was also supported by CIBER-BBN (Centro de Investigación Biomédica en Red, CB06/01/1021), ISCIII, Ministerio de Ciencia, Innovación y Universidades (MCIU) and European Regional Development Fund; and by Centro de Investigación Biomédica en Red CIBERINFEC (CB21/13/00044), ISCIII, MCIU, and Unión Europea – NextGenerationEU.

CRediT authorship contribution statement

Samuel Takvor-Mena: Writing – original draft, Methodology, Investigation, Formal analysis. **Andrea Barrios-Gumiel:** Writing – original draft, Methodology, Investigation, Formal analysis. **Carlos Rescalvo-Casas:** Writing – original draft, Methodology, Investigation, Formal analysis. **Felipe Pérez-García:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Matilde Alique:** Writing – review & editing, Supervision, Conceptualization. **Rafael M. Ramírez:** Writing – review & editing, Conceptualization. **Juan Soliveri:** Writing – review & editing, Methodology, Conceptualization. **Irene Heredero-Bermejo:** Writing – review & editing, Resources, Methodology, Formal analysis. **Ramón Pérez-Tanoira:** Writing – review & editing, Methodology, Investigation. **Javier Sánchez-Nieves:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Rafael Gómez:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

S.T.-M. and C.R.-C. acknowledge UAH for respective fellowships.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jiec.2026.01.025>.

Data availability

Data will be made available on request.

References

- [1] M. Castellanos, A. Somoza, Emerging clinically tested detection methods for COVID-19, *FEBS J.* 290 (2023) 3089–3104.
- [2] M.N. Emaus, M. Varona, D.R. Eitzmann, S. Hsieh, V.R. Zeger, J.L. Anderson, Nucleic acid extraction: Fundamentals of sample preparation methodologies, current advancements, and future endeavors, *TrAC Trends Anal. Chem.* 130 (2020) 115985.
- [3] T. Friedmann, R. Roblin, Gene Therapy for Human Genetic Disease? *Science* 175 (1972) 949–955.
- [4] J.A. Kulkarni, D. Witzigmann, S.B. Thomson, S. Chen, B.R. Leavitt, P.R. Cullis, R. van der Meel, The current landscape of nucleic acid therapeutics, *Nat. Nanotechnol.* 16 (2021) 630–643.
- [5] H. Zhu, H. Luo, R. Chang, Y. Yang, D. Liu, Y. Ji, H. Qin, H. Rong, J. Yin, Protein-based delivery systems for RNA delivery, *J. Controlled Release* 363 (2023) 253–274.
- [6] B. Wong, R. Birtch, R. Rezaei, T. Jamieson, M.J.F. Crupi, J. Djalio, C.S. Ilkow, Optimal delivery of RNA interference by viral vectors for cancer therapy, *Mol. Ther.* 31 (2023) 3127–3145.
- [7] Y. Qin, L. Ou, L. Zha, Y. Zeng, L. Li, Delivery of nucleic acids using nanomaterials, *Mol. Biomed.* 4 (2023) 48.
- [8] M. Mashal, N. Attia, S. Grijalvo, R. Eritja, G. Puras, J.L. Pedraz, Stability of polymeric cationic niosomes and their plasmid DNA-based complexes as gene delivery carriers, *Drug Deliv.* 30 (2023) 2219420.
- [9] M.D. Navarro, J. Carracedo, R. Ramirez, J.A. Madueño, A. Merino, M. Rodriguez, A. Martin-Malo, P. Aljama, Bacterial DNA prolongs the survival of inflamed mononuclear cells in haemodialysis patients, *Nephrol. Dial. Transplant.* 22 (2007) 3580–3585.
- [10] C.C. Szeto, B. Kwan, K.M. Chow, J. Kwok, K.B. Lai, P. Cheng, W.F. Pang, J. Kit-Chung Ng, M. Chan, L. Lit, C.B. Leung, P. Li, Circulating Bacterial-Derived DNA Fragment Level is a strong Predictor of Cardiovascular Disease in Peritoneal Dialysis patients, *PLoS One* 10 (2015) e0125162.
- [11] M. Zhang, Y. Cai, X. Zhong, W. Liu, Y. Lin, Z. Qiu, R. Liang, H. Wei, K. Wu, Q. Liu, Effects of cell-free DNA on kidney disease and intervention strategies, *Front. Pharmacol.* 15 (2024) 1377874.
- [12] J.W.M. Agar, K.A. Barraclough, Water use in dialysis: environmental considerations, *Nat. Rev. Nephrol.* 16 (2020) 556–557.
- [13] I. Yildiz, Applications of magnetic nanoparticles in biomedical separation and purification, *Nanotechnol. Rev.* 5 (2016) 331–340.
- [14] M. Magro, F. Vianello, Bare iron oxide nanoparticles: Surface tunability for biomedical, sensing and environmental applications, *Nanomaterials (basel, Switzerland)* 9 (2019) 1608.
- [15] K. Kalaitzidou, N. Maniotis, M. Pylotis, A. Lantás, T. Asimakidou, C. Virgiliou, K. Simeonidis, M. Mitakakis, Magnetically-assisted removal of selenium and molybdenum from water using iron oxyhydroxides, *J. Water Process Eng.* 58 (2024) 104915.
- [16] C. Tang, Z. He, H. Liu, Y. Xu, H. Huang, G. Yang, Z. Xiao, S. Li, H. Liu, Y. Deng, Z. Chen, H. Chen, N. He, Application of magnetic nanoparticles in nucleic acid detection, *J. Nanobiotechnol.* 18 (2020) 62.
- [17] I. Vilimová, I. Chourpa, S. David, M. Součá, K. Hervé-Aubert, Two-step formulation of magnetic nanoprobes for microRNA capture, *RSC Adv.* 12 (2022) 7179–7188.
- [18] A.I.S. van den Berg, C. Yun, R.M. Schiffelers, W.E. Hennink, Polymeric delivery systems for nucleic acid therapeutics: Approaching the clinic, *J. Controlled Release* 331 (2021) 121–141.
- [19] Alexander P. Majewski, Anja Schallon, Valérie Jérôme, Ruth Freitag, Axel H. E. Müller, Holger Schmalz, Dual-Responsive Magnetic Core-Shell Nanoparticles for Nonviral Gene Delivery and Cell Separation, *Biomacromolecules.* 13 (2012) 857–866.
- [20] M. Malkoch, S. García-Gallego, *Dendrimer Chemistry: Synthetic Approaches Towards complex Architectures*, 1st ed., Royal Society of Chemistry, Cambridge, 2020.

- [21] D. Shcharbin, E. Pedziwiatr, J. Blasiak, M. Bryszewska, How to study dendriplexes II: Transfection and cytotoxicity, *J. Controlled Release* 141 (2010) 110–127.
- [22] D. Shcharbin, A. Janaszewska, B. Klajnert-Maculewicz, B. Ziemia, V. Dzmitruk, I. Halets, S. Loznikova, N. Shcharbina, K. Milowska, M. Ionov, A. Shakhbazau, M. Bryszewska, How to study dendrimers and dendriplexes III, Biodistribution, Pharmacokinetics and Toxicity in Vivo, *Journal of Controlled Release* 181 (2014) 40–52.
- [23] T. Henser-Brownhill, L. Martin, P. Samangouei, A. Ladak, M. Apostolidou, B. Nagel, A. Kwok, In Silico Screening Accelerates Nanocarrier Design for Efficient mRNA delivery, *Adv. Sci.* (2024) e2401935.
- [24] F.J. de la Mata, R. Gómez, J. Cano, J. Sánchez-Nieves, P. Ortega, S. García-Gallego, Carbosilane dendritic nanostructures, highly versatile platforms for pharmaceutical applications, *WIREs Nanomed. Nanobiotechnol.* 15 (2023) e1871.
- [25] S. Quintana-Sánchez, A. Barrios-Gumiel, J. Sánchez-Nieves, J.L. Copa-Patiño, F. J. de la Mata, R. Gómez, Bacteria capture with magnetic nanoparticles modified with cationic carbosilane dendritic systems, *Biomaterials Adv.* 133 (2022) 112622.
- [26] D. Rincón-Montón, E. Hernández-Coroto, R. Lozano-García, J. Cano, F.J. De La Mata, R. Gómez, M.L. Marina, M.C. García, J. Sánchez-Nieves, Reusable and green approach to extract proteins with magnetic nanoparticles coated with carbosilane dendrimers: application to the recovery of whey proteins, *Int. J. Biol. Macromol.* 329 (2025).
- [27] A. Barrios-Gumiel, D. Sepúlveda-Crespo, J.L. Jiménez, R. Gómez, M.Á. Muñoz-Fernández, F.J. de la Mata, Dendronized magnetic nanoparticles for HIV-1 capture and rapid diagnostic, *Colloids Surf. B Biointerfaces* 181 (2019) 360–368.
- [28] M.K. Hadi, X. Wang, Y. Peng, S. Sangaraju, F. Ran, Functional Polymeric Membrane Materials: a Perspective from Versatile Methods and Modification to potential applications, *Polym. Sci. Technol.* 1 (2025) 366–412.
- [29] L. Jaber, A. Abushawish, O. Awayssa, A. Shanableh, M.A. Atieh, I.W. Almanassra, APTES-functionalized MoS₂@ZnO nanocomposite for ultrafiltration: Advanced characterization and oil/water separation performance, *Chem. Eng. J.* 518 (2025) 164604.
- [30] M. Vedhanayagam, A.S. kumar, B.U. Nair, K.J. Sreeram, Dendrimer-Functionalized Metal Oxide Nanoparticle-Mediated Self-Assembled Collagen Scaffold for Skin Regenerative Application: Function of Metal in Metal Oxides, *Appl Biochem Biotechnol.* 194 (2022) 266–290.
- [31] M. Sánchez-Milla, R. Gómez, J. Pérez-Serrano, J. Sánchez-Nieves, F.J. de la Mata, Functionalization of silica with amine and ammonium alkyl chains, dendrons and dendrimers: Synthesis and antibacterial properties, *Mater. Sci. Eng. C* 109 (2020) 110526.
- [32] P. Voparilová, Z. Šplíchal, P. Švec, P. Kulich, O. Malina, M. Otyepka, O. Zítka, J. Kudr, Amine-modified magnetic particles: an efficient tool for enhanced RNA extraction, *Sep. Purif. Technol.* 356 (2025) 129788.
- [33] N. Bali, S.J. Brennhag, M. Björås, S. Bandyopadhyay, A. Manaf, Optimized synthesis of polyacrylic acid-coated magnetic nanoparticles for high-efficiency DNA isolation and size selection, *RSC Adv.* 13 (2023) 2919–12912.
- [34] P. Oberacker, P. Stepper, D.M. Bond, S. Höhn, J. Focken, V. Meyer, L. Schelle, V. J. Sugrue, G. Jeunen, T. Moser, S.R. Hore, F. von Meyenn, K. Hipp, T.A. Hore, T. P. Jurkowski, Bio-On-Magnetic-Beads (BOMB): Open platform for high-throughput nucleic acid extraction and manipulation, *PLoS Biol.* 17 (2019) e3000107.
- [35] T.A. Kerr, M.A. Ciorba, H. Matsumoto, V.R.T. Davis, J. Luo, S. Kennedy, Y. Xie, A. Shaker, B.K. Dieckgraefe, N.O. Davidson, Dextran Sodium Sulfate Inhibition of Real-Time Polymerase Chain Reaction Amplification: a Poly-a Purification solution, *Inflamm. Bowel Dis.* 18 (2012) 344–348.
- [36] T. Strašák, J. Čermák, J. Sýkora, J. Horský, Z. Walterová, F. Jaroschik, D. Harakat, Carbosilane Metallo dendrimers with Titanocene Dichloride End groups, *Organometallics* 31 (2012) 6779–6786.
- [37] L. Madrid, C. Pinto-Díez, L. Muñoz-Moreno, M. García-Hernández, V.M. González, F.J. de la Mata, M.E. Martin, P. Ortega, Cationic Carbosilane Dendrimers for Apmnk2 Aptamer Transfection in Breast Cancer: An Alternative to Traditional Transfectants, *Macromol. Biosci.* 25 (2025) 2400327.
- [38] E. Fuentes-Paniagua, J. Sánchez-Nieves, J.M. Hernández-Ros, A. Fernández-Ezequiel, J. Soliveri, J.L. Copa-Patiño, R. Gómez, F.J. de la Mata, Structure-activity relationship study of cationic carbosilane dendritic systems as antibacterial agents, *RSC Adv.* 6 (2016) 7022–7033.