











Comparative Studies on Dispersion Characters of Titanium Dioxide (TiO₂) Nanoparticles for Rodent Toxicology Studies

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Abstract: This research aimed to find a suitable vehicle (media) for dispersing titanium dioxide nanoparticles (TiO₂ NPs) to investigate their toxicity in laboratory rodents. The TiO₂ NPs were suspended in different vehicles viz., distilled water, Tween 80 (0.1% v/v), normal saline, normal saline + Tween 80 (0.1% v/v) and carboxymethylcellulose (CMC; 0.1%, 0.2%, 0.3% or 0.4% w/v). The hydrodynamic diameters, zeta potential, and polydispersity index were measured by the dynamic light scattering (DLS) technique by employing a zeta sizer. The TiO₂ NPs in Tween 80 (0.1% v/v) and CMC (0.2% w/v) showed lesser hydrodynamic diameter compared to other vehicles. Further, TiO₂ NPs dispersed in CMC (0.1%, 0.2%, or 0.3% w/v) showed relatively better zeta potential in terms of better stability than other media examined. The polydispersity index of nanoparticles was relatively less for CMC: 0.1% w/v followed by CMC: 0.2% w/v. Thus, it is concluded that CMC (0.2% w/v) can be used as an ideal vehicle among the various media examined for delivery of TiO₂ NPs for toxicity testing in laboratory rodents.

Keywords: titanium dioxide (TiO₂) nanoparticles; hydrodynamic diameter; zeta potential; polydispersity index.

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1. Introduction

Nanoparticles (NPs) are organic or inorganic substances that range in size from 1 to 100 nm and can be of natural origin, produced by human activity, or engineered for specific purposes [1]. Nanotechnology involves the creation of engineered nanoparticles (ENPs) with

properties that distinguish them from their bulk counterparts, such as shape, size, charge, surface area to mass ratio, crystallinity, dissolution rate, reactive groups, and agglomeration or dispersion state [2,3].

Titanium dioxide is one of the most widely manufactured and consumed engineered nanoparticles (ENPs) in consumer products and research applications [4-8]. The TiO₂ NPs will unavoidably be released into the environment due to their large-scale manufacture, widespread usage, and unregulated disposal, posing a threat to individual organisms and ecosystems [9-17]. Therefore, it has become critical to determine the assessment of the risk and toxicity of NPs [18-20]. The physicochemical characterization of NPs is a crucial element of nanotoxicological investigations since the toxicity of NPs is strongly reliant on their size, shape, surface area, and surface charge [21-23]. The NPs, including TiO₂ tend to agglomerate into micrometer-sized coarse structures in aqueous media and thus exert different biological effects compared to well-dispersed NPs. Hence, NPs with coarse agglomerates in their dispersions are unsuitable for testing biological effects *in vivo* or *in vitro*. As a result, choosing the right vehicle is critical for determining the toxicological reaction to NPs. Therefore, the goal of the current study was to find an appropriate media to serve as a vehicle for toxicity testing of TiO₂ NPs in rodent toxicity testing protocols.

2. Materials and Methods

2.1. Chemicals.

Titanium dioxide [Titanium (IV) oxide] *anatase* (TiO₂; MW: 79.87 g.mol⁻¹) nanopowder (<25 nm particle size; 99.7% purity on trace metal basis) manufactured by Ms. Sigma-Aldrich, St. Louis, MO, USA and procured by Veterinary College, Shivamogga was employed. Analytical grade carboxymethylcellulose (CMC), Tween 80 were procured from Ms. S.d. Fine Chemicals Ltd., Mumbai, India. I.P grade normal saline was procured locally.

Titanium dioxide nanoparticles were suspended in different vehicles *viz.*, distilled water, Tween 80 (0.1% v/v), normal saline, normal saline + Tween 80 (0.1% v/v), CMC (0.1%, 0.2%, 0.3%, or 0.4% w/v). Each of the aforesaid TiO₂ NPs suspensions was subjected to ultrasonication for about 30 min to achieve proper dispersal.

2.2. Determination of size and zeta potential.

The hydrodynamic diameters, *zeta* potential, and polydispersity index of TiO₂ NPs in above-mentioned vehicles were measured by Dynamic Light Scattering (DLS) technique by employing *zeta* sizer (Nanopartica[®], HORIBA, SZ-100; Japan). The instrument is equipped with 10 mW or 100 mW laser beam at a wavelength of 532 nm. The surface charge of TiO₂ NPs was measured by an electrophoretic light scattering technique using a fold capillary cuvette (Folded capillary cell, DTS1070, Malvern, U. K). *Zeta* potential was measured with an electrode voltage of 3.9 V at 25 °C.

3. Results and Discussion

Many of the NPs employed in toxicology research is available in the form of powder. *In vitro* and *in vivo* research studies/experiments frequently necessitate effective dispersion of the powdered NPs in appropriate solutions. The van der Waals attractive forces cause the NPs to cluster and re-agglomerate with one other, resulting in biological activity change. To

correlate the physicochemical features of NPs with their hazardous potential, stable nanoparticle dispersions are required. The general requirement for preparing stable dispersion is to increase repulsive interactions between particles to prevent agglomeration [24]. To achieve this, ultrasonication is used as a repulsive force source, resulting in NPs dispersion in the vehicle and resistance to inter-particle attractive forces. The ability of ultrasonication to disperse NPs is due to acoustic cavitation. Acoustic cavitation creates a unique interaction of energy and substance, impairing NP conjugation and dispersing them in the stabilization process [25].

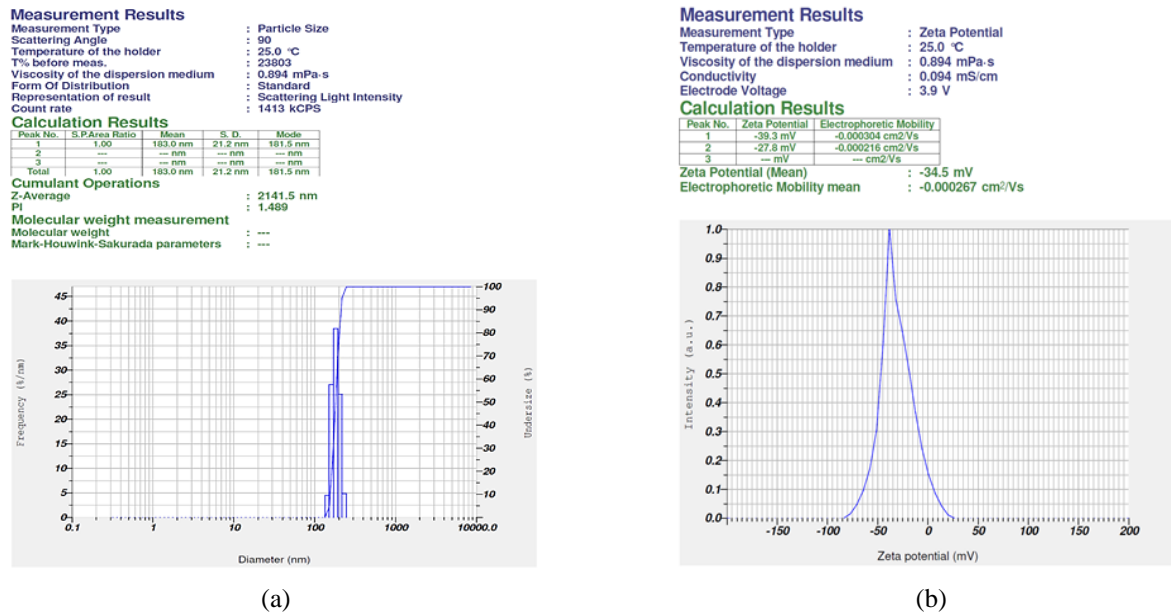


Figure 1. Hydrodynamic diameter (a) and Zeta potential (b) of TiO₂ NPs in distilled water.

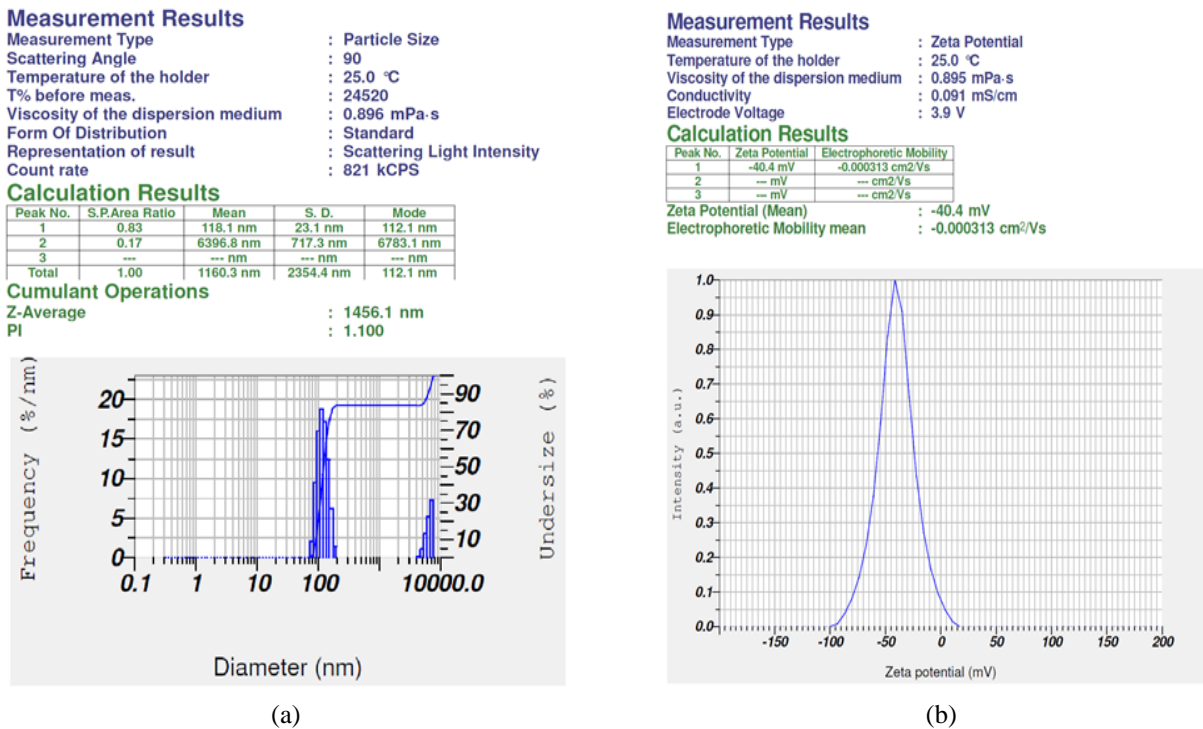


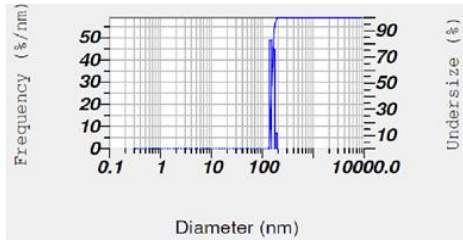
Figure 2. Hydrodynamic diameter (a) and Zetapotential (b) of TiO₂ NPs in Tween 80 (0.1% v/v)

Measurement Results
 Measurement Type : Particle Size
 Scattering Angle : 90
 Temperature of the holder : 25.0 °C
 T% before meas. : 28529
 Viscosity of the dispersion medium : 0.894 mPa·s
 Form Of Distribution : Standard
 Representation of result : Scattering Light Intensity
 Count rate : 52 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	153.5 nm	11.8 nm	150.8 nm
2	---	---	---	---
3	---	---	---	---
Total	---	153.5 nm	11.8 nm	150.8 nm

Cumulant Operations
 Z-Average : 27794.0 nm
 PI : 0.420



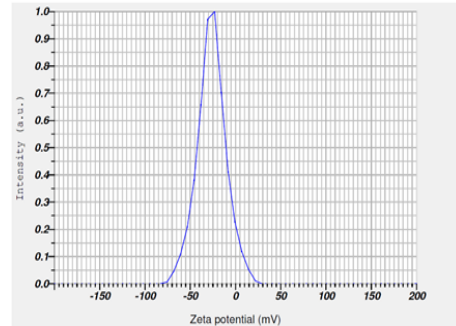
(a)

Measurement Results
 Measurement Type : Zeta Potential
 Temperature of the holder : 25.0 °C
 Viscosity of the dispersion medium : 0.894 mPa·s
 Conductivity : 0.415 mS/cm
 Electrode Voltage : 3.3 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-26.4 mV	-0.000205 cm ² /Vs
2	---	---
3	---	---

Zeta Potential (Mean) : -26.4 mV
 Electrophoretic Mobility mean : -0.000205 cm²/Vs



(b)

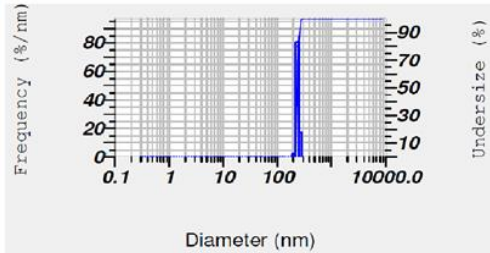
Figure 3. Hydrodynamic diameter (a) and Zeta potential (b) of TiO₂ NPs in normal saline.

Measurement Results
 Measurement Type : Particle Size
 Scattering Angle : 90
 Temperature of the holder : 25.0 °C
 T% before meas. : 23189
 Viscosity of the dispersion medium : 0.895 mPa·s
 Form Of Distribution : Standard
 Representation of result : Scattering Light Intensity
 Count rate : 2031 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	237.0 nm	12.3 nm	235.0 nm
2	---	---	---	---
3	---	---	---	---
Total	1.00	237.0 nm	12.3 nm	235.0 nm

Cumulant Operations
 Z-Average : 13748.8 nm
 PI : 3.199



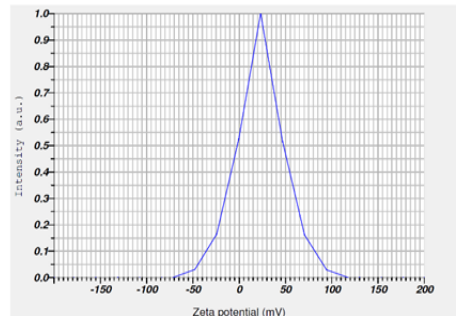
(a)

Measurement Results
 Measurement Type : Zeta Potential
 Temperature of the holder : 25.0 °C
 Viscosity of the dispersion medium : 16.894 mS/cm
 Conductivity : 1.1 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	23.0 mV	0.000178 cm ² /Vs
2	---	---
3	---	---

Zeta Potential (Mean) : 23.0 mV
 Electrophoretic Mobility mean : 0.000178 cm²/Vs



(b)

Figure 4. Hydrodynamic diameter (a) and Zetapotential (b) of TiO₂ NPs in normal saline + Tween 80 (0.1% v/v).

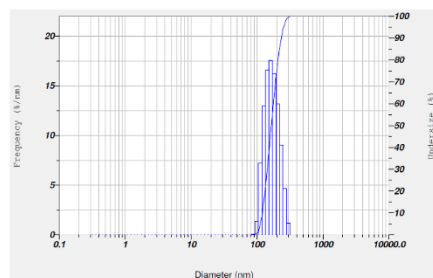
Measurement Results
 Measurement Type : Particle Size
 Scattering Angle : 90
 Temperature of the holder : 25.0 °C
 T% before meas. : 14032
 Viscosity of the dispersion medium : 0.894 mPa·s
 Form Of Distribution : Standard
 Representation of result : Scattering Light Intensity
 Count rate : 4131 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	171.1 nm	42.8 nm	160.9 nm
2	---	---	---	---
3	---	---	---	---
Total	1.00	171.1 nm	42.8 nm	160.9 nm

Cumulant Operations
 Z-Average : 187.7 nm
 PI : 0.239

Molecular weight measurement : ---
 Molecular weight : ---
 Mark-Houwink-Sakurada parameters : ---

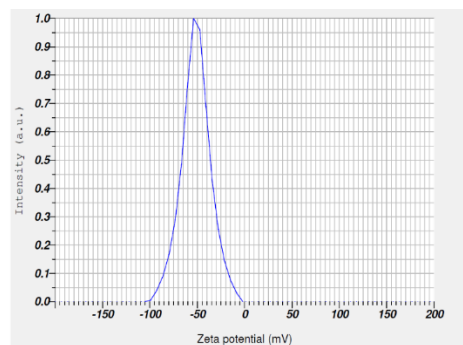


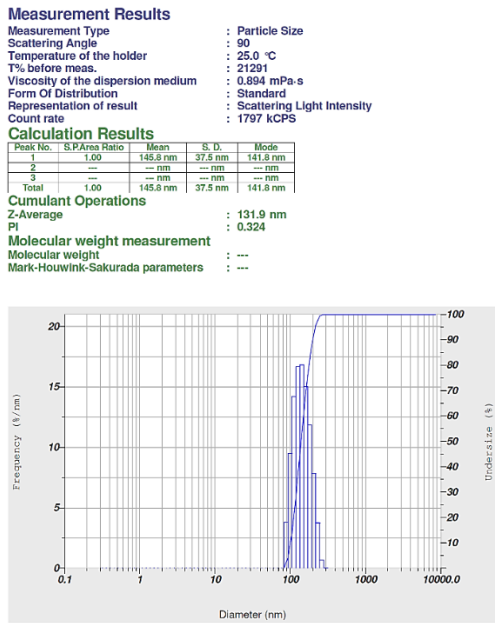
Measurement Results
 Measurement Type : Zeta Potential
 Temperature of the holder : 25.0 °C
 Viscosity of the dispersion medium : 0.894 mPa·s
 Conductivity : 0.075 mS/cm
 Electrode Voltage : 3.9 V

Calculation Results

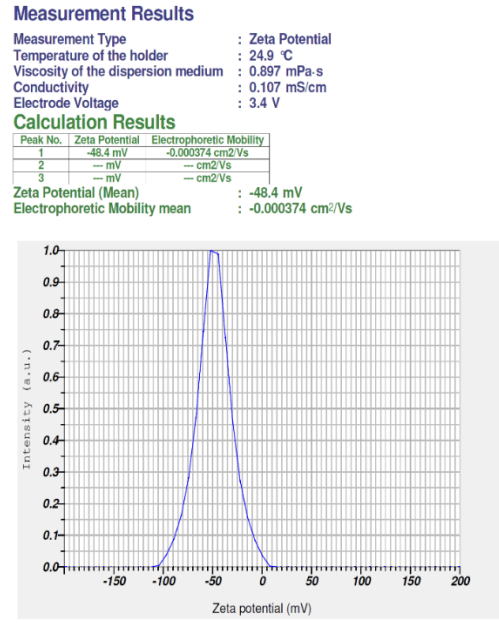
Peak No.	Zeta Potential	Electrophoretic Mobility
1	-51.3 mV	-0.000398 cm ² /Vs
2	---	---
3	---	---

Zeta Potential (Mean) : -51.3 mV
 Electrophoretic Mobility mean : -0.000398 cm²/Vs



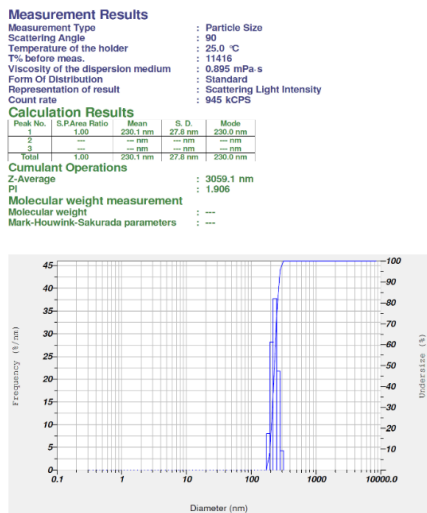


(a)

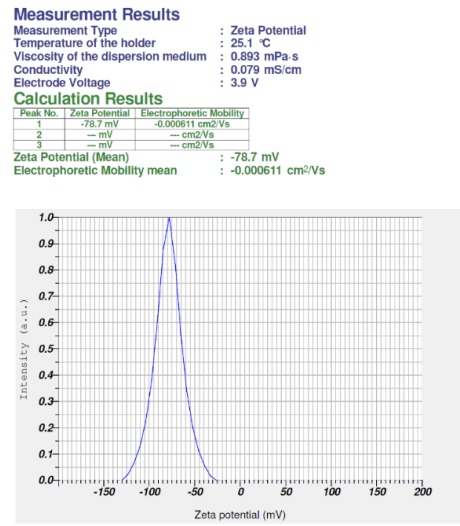


(b)

Figure 6. Hydrodynamic diameter (a) and Zetapotential (b) of TiO₂ NPs in CMC (0.2%).

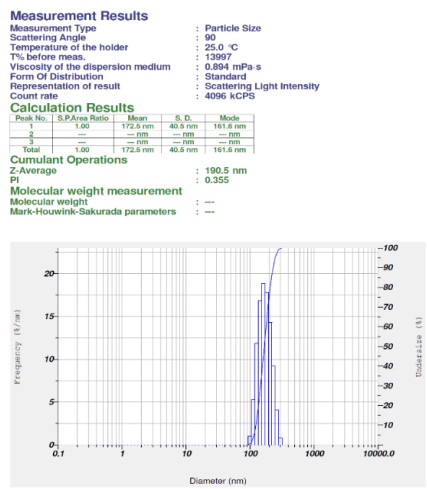


(a)

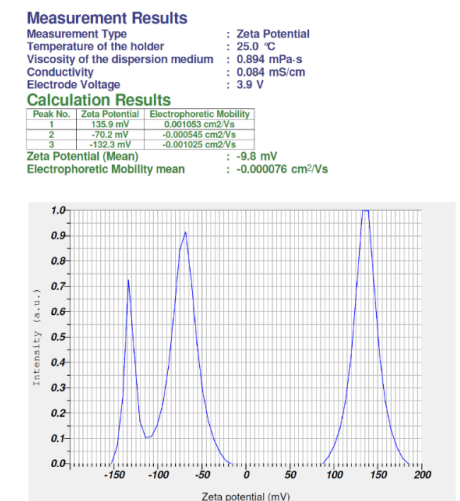


(b)

Figure 7. Hydrodynamic diameter (a) and Zetapotential (b) of TiO₂ NPs in CMC (0.3%).



(a)



(b)

Figure 8. Hydrodynamic diameter (a) and Zetapotential (b) of TiO₂ NPs in CMC (0.4%).

The sonication time for TiO₂ NPs suspension in various vehicles ranged from 10 min to an hour or more [26-32]. In the present study, sonication of TiO₂ NPs suspensions was carried out for 30 min. In an experiment, Kobayashi *et al.* [30] dispersed TiO₂ NPs in disodium hydrogen phosphate (0.2% w/v) and sonicated different volumes for 30 or 120 min, and they reported that 30 min and 10 ml were optimal for sonication of TiO₂ NPs suspensions. Further, they reported similar particle sizes for 30 and 120 min sonication.

The DLS method is considered a precise and efficient tool for nanoparticle measurement. The 'Brownian movement' of the particle is used to measure particle size using DLS analysis, which involves measuring dynamic fluctuations in light-scattering intensity. This method yields a particle's hydrodynamic diameter [33].

The hydrodynamic diameter of TiO₂ NPs in distilled water, Tween 80 (0.1% v/v), normal saline, normal saline + Tween 80 (0.1% v/v), CMC: 0.1%, 0.2%, 0.3% and 0.4% w/v was 181.5 nm (Fig. 1a), 112.1 nm (Fig. 2a), 150.8 nm (Fig. 3a), 235.0 nm (Fig. 4a), 160.9 nm (Fig. 5a), 141.8 nm (Fig. 6a), 230.0 nm (Fig. 7a) and 161.6 nm (Fig. 8a), respectively. Among these, the hydrodynamic diameter of TiO₂ NPs in Tween 80 (0.1% v/v) and CMC (0.2% w/v) was found to be less compared to other vehicles examined. In a study, Fadda *et al.* [31] reported a similar hydrodynamic diameter of TiO₂ NPs suspension in CMC (1% w/v). In another study, a similar result was reported by Zhang *et al.* [25]. They reported a higher hydrodynamic diameter of TiO₂ NPs in distilled water but reported lesser size with stabilizers like CMC (1% w/v), Hydroxypropyl methylcellulose (0.5%). Hu *et al.* [34] observed 208 and 330 nm diameter TiO₂ NPs in Hydroxypropyl methylcellulose solvent after incubating for 12 and 24 h, respectively.

Polymeric dispersion stabilizers play a role in nanoparticle dispersing. The CMC might have acted as stabilizers to reduce particle aggregation in suspension by reducing hydrodynamic diameter. In contrast to the above result, Fadoju *et al.* [29] and Grissa *et al.* [32] reported a larger hydrodynamic diameter for TiO₂ NPs suspension in distilled water. Lee *et al.* [35] reported 341.5 nm as the hydrodynamic diameter of TiO₂ NPs in deionized water.

The electric potential that a nanoparticle creates in a solution due to the formation of a double electrical layer is known as the *zeta* potential. The *zeta* potential is used to measure a nanoparticle's surface charge and stability [36]. The amount of *zeta* potential in a dispersion media shows the degree of electrostatic repulsion between adjacent, similarly charged particles. The charged particles with *zeta* potential above +30 mV or below -30 mV are thought to have enough electrostatic repulsion to ensure good colloidal stability [37]. When the *zeta* potential is close to neutral, the dispersion becomes less stable, making agglomeration/aggregation more likely. In the present study, the TiO₂ NPs were negatively charged in all the vehicles except in normal saline + Tween 80 (0.1% v/v). Among studied vehicles, TiO₂ NPs in CMC (0.1%, 0.2% and 0.3% w/v) showed better *zeta* potential of -51.3 mV (Fig. 5b), -48.4 mV (Fig. 6b) and -78.7 mV (Fig. 7b), respectively indicating better stability compared to other dispersing media. In different studies, Meena *et al.* [28], Fadda *et al.* [31], and Grissa *et al.* [32] reported negatively charged *zeta* potential for TiO₂ NPs. On the other hand, Hu *et al.* [34] and Lee *et al.* [35] reported positively charged *zeta* potential for TiO₂ NPs.

The nanoparticle solution's average homogeneity is calculated using the polydispersity index. When the size distribution of the particles in the sample is large, then the polydispersity index value is high. The polydispersity index scale is '0' to 1, with '0' indicating monodisperse and '1' indicating polydisperse [38]. The polydispersity index can also reveal nanoparticle agglomeration and the surface modifications in the sample efficiently [39]. In the present study,

the polydispersity index of TiO₂ NPs in distilled water, Tween 80 (0.1% v/v), normal saline, normal saline + Tween 80 (0.1% v/v), CMC (0.1%, 0.2%, 0.3% or CMC 0.4% w/v) was 1.489, 1.100, 0.420, 3.199, 0.239, 0.324, 1.906 and 0.355, respectively (Table: 1 and Fig. 1a to Fig. 8a). Among the various vehicles tested, the polydispersity index of TiO₂ NPs was less for CMC: 0.1% w/v followed by CMC: 0.2% w/v compared to other vehicles, indicating relatively less polydispersity of TiO₂ NPs when compared to other vehicles tested.

Table 1. Size distribution, surface charge, and polydispersity index of TiO₂ NPs in different vehicles

Vehicles	Hydrodynamic diameter (nm)	Zeta potential (mV)	Polydispersity index
Distilled water	181.5	-34.5	1.489
Tween 80 (0.1% v/v)	112.1	-40.4	1.100
Normal saline	150.8	-26.4	0.420
Normal saline+ Tween 80 (0.1% v/v)	235.0	+23.0	3.199
CMC (0.1% w/v)	160.9	-51.3	0.239
CMC (0.2% w/v)	141.8	-48.4	0.324
CMC (0.3% w/v)	230.0	-78.7	1.906
CMC (0.4% w/v)	161.6	-9.8	0.355

4. Conclusions

The dispersion of TiO₂ NPs in a suitable vehicle is of utmost importance for toxicity testing studies in rodents. The current study indicates that carboxy methylcellulose (CMC) (0.2% w/v) can be an ideal vehicle for the delivery of titanium dioxide (TiO₂) nanoparticles in rodent toxicological studies.

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Conflicts of Interest

The authors declare no conflict of interest.

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