Evaluation of Nanodispersion of Iron Oxides Using Various Polymers

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Tanaka, *et al.*: Effect of Various Polymers on Nanoparticulation

In order to create Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ nanoparticles, various polymers were used as dispersing agents, and the resulting effects on the dispersibility and nanoparticulation of the iron oxides were evaluated. It was revealed that not only the solution viscosity but also the molecular length of the polymers and the surface tension of the particles affected the dispersibility of Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles. Using the dispersing agents 7.5% hydroxypropylcellulose-SSL, 6.0% Pharmacoat 603, 5.0% and 6.5% Pharmacoat 904 and 7.0% Metolose SM-4, Fe_2O_3 nanoparticles were successfully fabricated by wet milling using Ultra Apex Mill. $Fe_2O_3 \cdot H_2O$ nanoparticles could also be produced using 5.0% hydroxypropylcellulose-SSL and 4.0 and 7.0% Pharmacoat 904. The index for dispersibility developed in this study appears to be an effective indicator of success in fabricating nanoparticles of iron oxides by wet milling using Ultra Apex Mill.

Key words: Dispersibility, iron oxide, nanoparticle, polymer, wet-milling

The colouring agents used as pharmaceutical excipients are classified into inorganic and organic substances, and organic colouring agents are further divided into natural and synthetic substances. Because organic colouring agents are soluble in water and/or organic solvents, the colour palette of the resulting tablets or capsules is clear and the printed marks are clean-cut. However, the natural colouring agents tend to have poor stability, and although coal-tar colours are typically used as synthetic colouring agents, the authorised use of tar colours is limited to only certain countries. Among inorganic substances, titanium dioxide and iron oxide are generally used as colouring agents. Although the use of inorganic colouring agents is generally allowed worldwide, the colour palette of the resulting tablets or capsules and the printed marks are not clear because the inorganic colouring agents are not soluble in water and/or organic solvents.

Iron oxides are very important materials in various industries and are widely used as inorganic dyes, food additives and pigments for cosmetics[1,2]. There are various kinds of iron oxides such as Fe_2O_3, $Fe_2O_3 \cdot H_2O$, FeO and Fe_3O_4, and these iron oxides are synthesised by various methods[3-5]. Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ are used as red and yellow pigments[6], respectively, and Fe_2O_3 has good properties owing to its nontoxicity, chemical stability and low cost[2]. The use of iron oxides as colouring agents for medical inks is considered one of the most effective ways to avoid the problems derived from the use of tar colours and organic solvents. However, it is difficult to uniformly disperse iron oxide particles in a water solvent because of the aggregation and sedimentation of the particles; the clogging of the particles at the nozzle of an ink-jet printer also creates problems with the use of iron oxide as ink.

According to the Stokes equation, the sedimentation rate of particles decreases in proportion to the decrease in the square of particle sizes and the increase in the viscosity of the solvent[7]. In other words, the dispersibility of iron oxide particles in water can be improved by adding polymers to a solvent (thus increasing the viscosity) and milling the particles (thus decreasing the size). Clogging problems that exist with ink-jet printers could also be solved by decreasing particle size.

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To enhance the capabilities of particles, many kinds of nanoparticulation techniques for various materials have been reported[2,8-10]. However, nanoparticles tend to aggregate owing to their high surface energy, thereby inducing phase separation[11]. Creating a stable nanosuspension of iron oxides is considered to be quite effective in order to utilise iron oxides for medical inks. Moreover, it enables smoother characters and logos to be printed on tablets or capsules without roughness. The usual approach for minimising particle aggregation is by adding dispersing agents such as polymers and surfactants. Various polymers are used for the stabilisation of particles, such as methylcellulose (MC), hydroxypropylcellulose (HPC) and hypermellose (HPMC). These polymers provide sufficient solution viscosity or steric barriers to inhibit contact between the coated particles[12]. However, the inhibitory effects of particle aggregation are considerably different among each substance and polymer.

In this study, in order to create a stable medical ink from iron oxides, we investigated the effects of various polymers on the dispersibility and nanoparticulation of Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ using Ultra Apex Mill.

MATERIALS AND METHODS

Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ were provided by Kishi Kasei Ltd. Yokohama, Japan. Nisso HPC-SSL (MW: 40 000), -SL (MW: 100 000), -L (MW: 140 000), and -H (MW: 910 000) were purchased from Nippon Soda Co., Ltd., Tokyo, Japan. HPMCs such as TC-5E (Pharmacoat 603), TC-5M (Pharmacoat 645), TC-5R (Pharmacoat 606), 60SH-4000, 60SH-10000 and SB4 (Pharmacoat 904) and MCs such as Metolose SM-4 and SM-4000 were purchased from Shin-Etsu Chemical Co., Ltd., Tokyo, Japan. Pluronic® F-68 and F-108 were purchased from Adeca Alcohol Delivery Company, Japan. Plasdone® S-630 was purchased from Wako Pure Chemical Industries Ltd., Japan. All other reagents were analytical-grade commercial products.

Measurement of viscosity:

Various polymers were dissolved in distilled water at various concentrations (w/w%). The viscosities of these solutions were measured using a viscometer with an S61 spindle (LVDV-I Prime, Brookfield Engineering Laboratories, Inc., Middleboro, Massachusetts, USA) at 60 rpm (11/s) and 20°.

Evaluation of dispersibility of Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles:

Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ were suspended at 10 w/w% into the various polymer solutions by a homogeniser (3000 rpm (50/s), 30-45 s; HM-300, Hsiangtai Machinery Industry Co., Ltd., New Taipei, Taiwan). The suspensions were then centrifuged for 5 min using a centrifuge at 5590×*g* of relative centrifugal force (RCF). Each supernatant was diluted to one-tenth with distilled water, and each transmittance (%) was determined at 500 nm by a spectrophotometer (UVmin-1240, Shimadzu Corporation, Kyoto, Japan). The dispersibility of Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles was evaluated in terms of the index for dispersibility (IFD), as defined in the following equation: IFD=100 − transmittance. The RCF was calculated by the following equation: $\text{RCF} (\times g)=1118\times R\times N^2\times 10^{-8}$, where R is the radius of the centrifuge (cm) and N is the rotational speed (rpm).

Measurement of penetration length of various polymer solutions into Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles in glass tube:

Fe_2O_3 (2.2 g) and $Fe_2O_3 \cdot H_2O$ (0.5 g) particles were placed in a glass tube (inner diameter: 6 mm), the other end of which was sealed with a paper filter. The densities of the particles were adjusted to 0.52 and 0.59 g/cm^3, respectively, by tapping. Then, 1 and 0.3 ml of the various polymer solutions was added through the top of the glass tube filled with Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles, respectively. After 3 h for Fe_2O_3 and 1 h for $Fe_2O_3 \cdot H_2O$, the penetration length of various polymer solutions into Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ was measured as the index for surface tension.

Scanning electron microscopy analysis:

A scanning electron microscope (SEM, S-4100, Hitachi Ltd., Tokyo, Japan) operating at 5 keV was used to determine the shape of the iron oxides particles. Prior to SEM analyses, the iron oxides particles were dispersed onto a carbon-tape-coated aluminium stub and then coated with gold.

Nanoparticulation of iron oxide particles using the Ultra Apex Mill:

Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles were suspended at about 15 w/w% into the various polymer solutions, and wet milling was conducted using Ultra Apex Mill, which is a novel wet-mill instrument using Centri separator instead of a screen type to isolate a wet-milled substrate from beads[13-15]. The procedure has been reported in detail previously[13-15]. The milling chamber of the Ultra Apex Mill was filled with 500 g of fine zirconia beads (diameter: 0.05 mm). The beads were stirred by a rotor pin (rotation speed: 10.0 m/s); the iron oxide suspensions with various polymers were then poured into a slurry tank. The iron oxide particles were milled in the milling chamber by impact with the zirconia beads. The milled iron oxides were separated from the zirconia beads by a Centri separator and the separated milled suspension was collected. The particle sizes of the iron oxides in the various milled suspensions were immediately measured.

Measurement of particle size:

The particle size distribution in the various iron oxide suspensions was measured using a laser scattering analyser (LSA, LA-950, Horiba, Japan), and the mean particle size was calculated.

RESULTS

IFD values of Fe_2O_3 particles and viscosities of the various polymer solutions:

The IFD values of the Fe_2O_3 particles were plotted against the viscosities of the various polymer solutions (fig. 1). The value associated with 5.0% Pharmacoat 606 was the highest (100) and the IFD value related to 19% Plasdone S-630 was the lowest (0.00) among the various IFD values. According to the Stokes equation, the IFD values of Fe_2O_3 particles in the polymer solutions with higher viscosity are considered to be high as compared with those with lower viscosity. Although the solutions with viscosities greater than 20 MPas seemed to provide better IFD values (greater than 50), good correlation was not obtained in this range ($R^2=0.0002$). For example, the IFD values associated with 0.5% HPC-H and 20% Pluronic F-68 were much lower, regardless of the fact that their viscosities were greater than 20 MPas.

Relationships between IFD values of the iron oxides and molecular length of each polymer:

In order to evaluate the effect of the molecular length of each polymer on the IFD values, the molecular lengths of HPC, HPMC and MC were calculated from each mean degree of polymerisation (DPw), using data provided by Nippon Soda Co., Ltd., and Shin-Etsu Chemical Co., Ltd., and the monomer molecular size of glucose by assuming that these polymers are straight chains (Table 1). The minimum energy of glucose was calculated by the molecular mechanics (MM2) program in Chem 3D® Pro ver. 8.0, and the glucose size from oxygen of 1C to 4C was estimated to be 0.42 nm. The relationships between the IFD values and the lengths are shown in figs. 2 and 3. The IFD values of Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ evaluated from each polymer solution with almost the same viscosity (20.5-27.3 and 10.5-15.4 MPas for Fe_2O_3 and $Fe_2O_3 \cdot H_2O$, respectively) were used to avoid the influence of viscosity on the relationships. In Fe_2O_3 (fig. 2a-c), the IFD in solution of HPC-SSL, Pharmacoat 645, Pharmacoat 606 and Pharmacoat 904, all of which have shorter lengths,

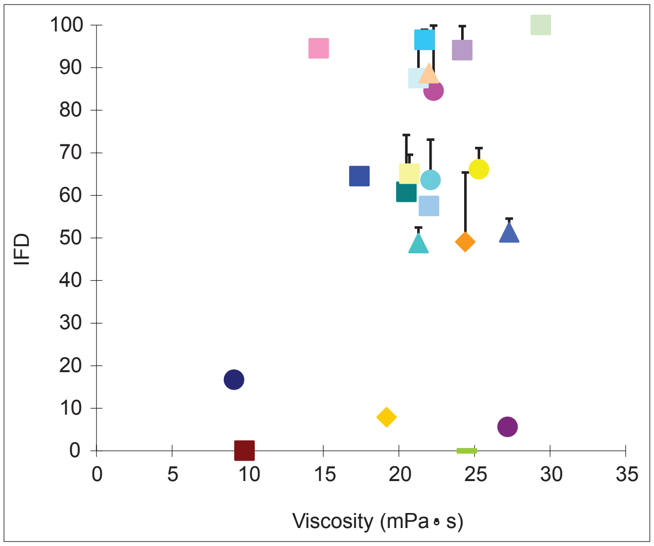

Fig. 1: Relationships between IFD values of Fe_2O_3 particles and viscosities of the various polymer solutions.
The figures in the parentheses indicate the concentration (%) of each polymer. IFD values related to HPC-SSL (5.0), Pharmacoat 603 (5.0), Pharmacoat 645 (5.0), Pharmacoat 606 (5.0) and Pharmacoat 904 (5.0) are *N*=1. The others are *N*=3. ● HPC-SSL (5.0), ● HPC-SSL (8.0), ● HPC-SL (5.0), ● HPC-L (3.5), ● HPC-H (0.5), ■ Pharmacoat 603 (5.0), ■ Pharmacoat 603 (7.0), ■ Pharmacoat 645 (5.0), ■ Pharmacoat 645 (5.5), ■ Pharmacoat 606 (4.0), ■ Pharmacoat 606 (5.0), ■ 60SH-4000 (0.45), ■ 60SH-10000 (0.45), ■ Pharmacoat 904 (5.0), ■ Pharmacoat 904 (5.5), ▲ Metolose SM-4 (5.0), ▲ Metolose SM-4 (5.5), ▲ SM-4000 (0.45), — Plasdone S-630 (19.0), ◆ Pluronic -68 (20), ◆; Pluronic F-108 (14.0).

TABLE 1: MEAN DEGREE OF POLYMERISATION AND MOLECULAR LENGTH OF THE VARIOUS POLYMERS

Polymers	Mean degree of polymerisation (DPw)	Molecular length (nm)
HPC-SSL	126	52.9
HPC-SL	310	130.2
HPC-L	433	181.9
HPC-H	2821	1184.8
Pharmacoat 603	79	33.2
Pharmacoat 645	112	47.0
Pharmacoat 606	175	73.5
60SH-4000	1323	555.7
60SH-10000	1828	767.8
Pharmacoat 904	100	42.0
Metolose SM-4	100	42.0
SM-4000	1500	630.0

Each polymer was assumed straight chain

showed high values ranging from 84.6 to 96.5. As the lengths increased, the IFD values tended to decrease, and the IFD value associated with 0.5% HPC-H with the longest length had the lowest IFD value (5.6). In case of $Fe_2O_3·H_2O$ (fig. 3a-c), the IFD values also decreased as the polymer lengths increased. The IFD values in solution of HPC-SSL and Pharmacoat 904 showed relatively high values of 78.9 and 75.5, respectively, whereas those of HPC-H, 60SH-4000, and 60SH-10000 were less than 1.

Relationships between IFD values of the iron oxides and the penetration lengths of each polymer solution into Fe_2O_3 and $Fe_2O_3·H_2O$ particles filled in glass tube:

The penetration length was measured as an index for surface tension. To avoid the influence of the viscosity of the polymer solution on the penetration length, the viscosity was constantly adjusted from 18.1 to 20.5 MPas (Table 2). The penetration length of the 6.5% Pharmacoat 603 solution into Fe_2O_3 showed the highest value (9.9 cm), followed by that of 7.5% HPC-SSL (9.7 cm) and Pharmacoat 904 (9.6 cm). The IFD of Fe_2O_3 in these polymer solutions showed a moderate value in 6.5% Pharmacoat 603 (60.8) and high values in both 7.5% HPC-SSL and Pharmacoat 904 (84.6 and 94.1, respectively). In the case of $Fe_2O_3·H_2O$, the penetration lengths of 7.5% HPC-SSL, 5.0% Pharmacoat 904 and 5.0% Pharmacoat 645 were greater than 3.0 cm and the IFD values related to these polymers were also high (59.9-78.9). Then, the regression lines between the IFD values of the iron oxides and the penetration lengths were calculated (figs. 4 and 5). In Fe_2O_3, the correlation coefficients were 0.931 and 0.349 for HPC and HPMC, respectively (fig. 4b and c, respectively). Although a good correlation was observed in HPC, a negative slope was obtained in HPMC. Overall,

TABLE 2: IFD VALUES OF FE_2O_3 AND $FE_2O_3 • H_2O$ (*N*=1) AND PENETRATION LENGTH OF EACH POLYMER SOLUTION (*N*=3)

Polymers (%)	Viscosity (MPas)	IFD		Penetration length (cm±SD)	
		Fe_2O_3	$Fe_2O_3•H_2O$	Fe_2O_3	$Fe_2O_3•H_2O$
HPC-SSL (7.5)	18.7	84.6	78.9	9.7±0.79	3.5±0.05
HPC-SL (5)	20.4	66.1	31.1	9.0±0.52	2.8±0.25
HPC-L (3.5)	20.4	63.6	8.8	9.5±0.21	2.7±0.24
HPC-H (0.45)	20.5	5.6	0.1	7.6±0.28	2.1±0.00
Pharmacoat 603 (6.5)	20.3	60.8	54.4	9.9±0.99	2.6±0.33
Pharmacoat 645 (5.0)	19.1	96.5	59.9	8.8±0.39	3.0±0.12
Pharmacoat 606 (4.0)	18.1	87.5	29.0	8.5±0.92	2.9±0.16
Pharmacoat 904 (5.0)	18.5	94.1	75.5	9.6±0.21	3.2±0.22

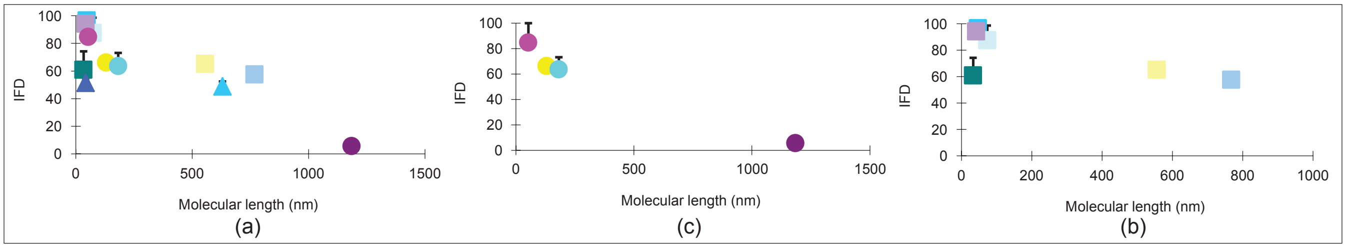

Fig. 2: Relationships between IFD values of Fe_2O_3 and molecular length of each polymer.
The figures in the parentheses indicate the concentration (%) of each polymer. a: all the polymers, b: HPC, c: HPMC; HPC-SSL (8.0), ● HPC-SL (5.0), ● HPC-L (3.5), ● HPC-H (0.5), ■ Pharmacoat 603 (7.0), ■ Pharmacoat 645 (5.5), ■ Pharmacoat 606 (4.0), ■ 60SH-4000 (0.45), ■ 60SH-10000 (0.45), ■ Pharmacoat 904 (5.5), ▲ Metolose SM-4 (5.5), ▲ SM-4000 (0.45).

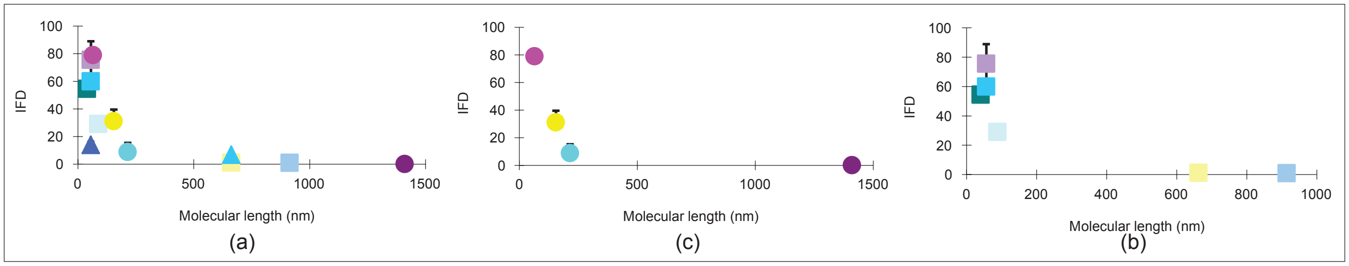

Fig. 3: Relationships between IFD values of $Fe_2O_3•H_2O$ and molecular length of each polymer.
The figures in the parentheses indicate the concentration (%) of each polymer. The IFD values of HPC-H (0.35) and Pharmacoat 603 (5.0) are N=1. The others are N=3. a: all the polymers, b: HPC, c: HPMC. ● HPC-SSL (6.0), ● HPC-SL (3.5), ● HPC-L (2.5), ● HPC-H (0.35), ■ Pharmacoat 603 (5.0), ■ Pharmacoat 645 (4.0), ■ Pharmacoat 606 (3.0), ■ 60SH-4000 (0.3), ■ 60SH-10000 (0.25), ■ Pharmacoat 904 (4.0), ▲ Metolose SM-4 (4.0), ▲ SM-4000 (0.35); N=3.

the correlation coefficient (R^2=0.318) was low (fig. 4a). In $Fe_2O_3 \cdot H_2O$, a relatively good correlation (R^2=0.698) between IFD values and the penetration lengths of all the polymer solutions was observed (fig. 5a). The correlation coefficient was high in HPC (R^2 = 0.889) and low in HPMC (R^2 = 0.223).

Particle morphology:

The morphological characteristics of the Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles were analysed using SEM (fig. 6). The SEM micrographs of the Fe_2O_3 particles revealed that nanosized particles (about 100-300 nm) were aggregated (fig. 6a). In the case of the $Fe_2O_3 \cdot H_2O$ particles, the morphology included porous aggregates composed of many micro-sized crystals having needle-like structures (fig. 6b).

Particle size measurement:

The mean sizes of the Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles with and without polymers were measured using a LSA (Table 3). The sizes of the Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ particles were 32.8 and 41.8 μm, respectively. After adding the 7.5% HPC-SSL, 6.0% Pharmacoat 603, 5.0% and 6.5% Pharmacoat 904 and 7.0% Metolose SM4 to Fe_2O_3 followed by wet milling using the Ultra Apex Mill, the particles became nanosized particles with sizes ranging from 0.45 to 0.76 μm. However, the other Fe_2O_3 particle sizes (milled with 0.75 or 0.90% HPC-H or 4.0% Pharmacoat 603) did not reach the nanometer range (1.12-1.59 μm). The $Fe_2O_3 \cdot H_2O$ particle size in the suspensions was 0.26-0.91 μm after milling with 5.0% HPC-SSL and with 4.0 and 7.0% Pharmacoat 904, but the particles were much larger when milled with 3.5% HPC-SSL (2.21 μm).

DISCUSSION

In order to develop a medical ink made from Fe_2O_3 and $Fe_2O_3 \cdot H_2O$ nanoparticles, the effects of various polymers on the dispersibility and nanoparticulation were investigated. According to the Stokes equation, the sedimentation rate of particles decreases inversely with the viscosity of the solvent[7]. We therefore

TABLE 3: MEAN PARTICLE SIZES OF FE_2O_3 AND $FE_2O_3 \cdot H_2O$ IN VARIOUS SUSPENSIONS AFTER OR BEFORE WET-MILLING

Iron oxide	Polymers (%)	Mean particle size (◦m)
Fe_2O_3		32.8
	HPC-SSL (7.5)	0.45
	HPC-H (0.75)	1.59
	HPC-H (0.90)	1.37
	Pharmacoat 603 (4.0)	1.12
	Pharmacoat 603 (6.0)	0.75
	Pharmacoat 904 (5.0)	0.76
	Pharmacoat 904 (6.5)	0.59
	Metolose SM-4 (7.0)	0.70
$Fe_2O_3 \cdot H_2O$		41.8
	HPC-SSL (3.5)	2.21
	HPC-SSL (5.0)	0.91
	Pharmacoat 904 (4.0)	0.51
	Pharmacoat 904 (7.0)	0.26

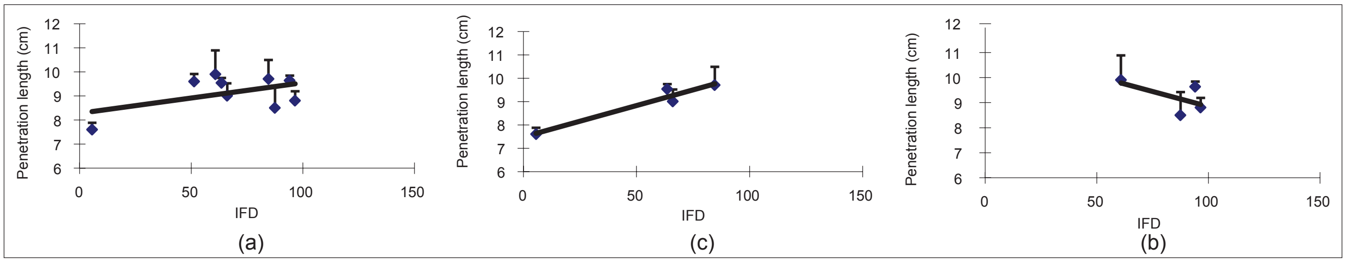

Fig. 4: Reationships between IFD values of Fe_2O_3 and penetration lengths of each polymer solution.
a: All the polymers, b: HPC, c: HPMC. Correlation coefficients (R^2) in figs. a, b and c were 0.318, 0.931 and 0.349, respectively.

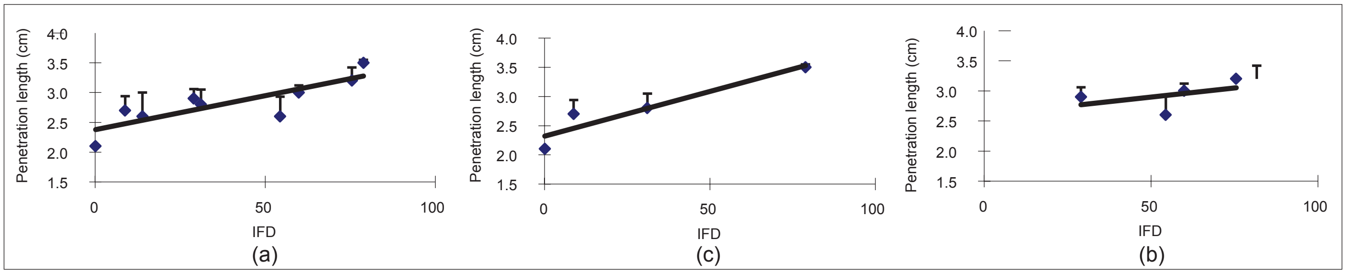

Fig. 5: Relationships between IFD values of $Fe_2O_3 \cdot H_2O$ and penetration lengths of each polymer solution.
a: all the polymers, b: HPC, c; HPMC. Correlation coefficients (R^2) in figs. a, b and c were 0.698, 0.889 and 0.223, respectively; (*N*=3).

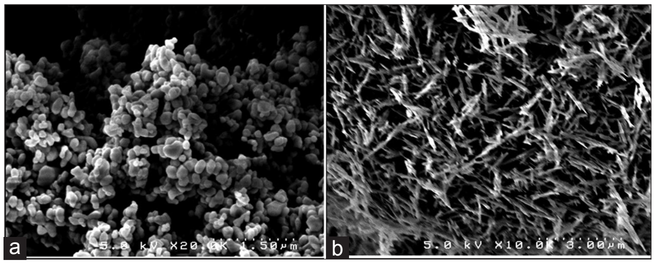

Fig. 6: SEM micrographs of particles
Scanning electron microscopic pictures of a: Fe_2O_3 and b: $Fe_2O_3 \bullet H_2O$ particles

first compared the viscosities of the various polymer solutions to the IFD values of the Fe_2O_3 particles (fig. 1). The Fe_2O_3 particles showed a tendency toward relatively high dispersibilities in solutions with viscosities of more than 20 MPas, but the correlation was not good among them ($R^2=0.0002$). These results indicate that not only the solution viscosity but also other factors such as the molecular length of the polymers and the surface tension on the particles have an impact on the dispersibility of Fe_2O_3 particles.

The relationship between the IFD values and the molecular lengths of HPMC, HPC and MC was evaluated under the condition that the viscosities of the polymer solutions were almost constant. Although these polymers do not generally form a straight chain and they are partially self-associated by nonspecific bindings such as hydrogen bonding, it is considered that the rough molecular lengths of these polymers could be estimated from the monomer glucose size. The IFD values increased with decreasing length of the polymers in both Fe_2O_3 and $Fe_2O_3{\cdot}H_2O$ (figs. 2 and 3). It is generally known that adding polymers inhibits aggregation of particles by coating substrates and providing steric barriers to hinder contacts between polymer-coated particles[16-19]. Under the constant viscosity of the polymer solutions, the polymer concentration (w/w%) decreased with increasing molecular length of each polymer (figs. 2 and 3), indicating that the mole number is much smaller in longer-length polymers. The reason for this may be due to insufficient coating of longer-length polymers onto the Fe_2O_3 and $Fe_2O_3{\cdot}H_2O$ particles because of the low mole number.

Next, the penetration length of various polymers for Fe_2O_3 and $Fe_2O_3{\cdot}H_2O$ was measured as an index for particle surface tension (Table 2, figs. 4 and 5). Wettability on the particle surface is also an important determinant for inhibiting particle aggregation[11,20,21]. Polymers are known to improve the wettability of hydrophobic substrates following their adsorption onto the particle surface by reducing the solid/liquid interfacial tension[22-25]. However, the IFD values of Fe_2O_3 did not correlate with the penetration length in the case of the HPMC solution. This may be due to the relatively lesser interaction of the Fe_2O_3 surface with HPMC molecules. Further investigation is needed for this. The penetration length of 6.5% Pharmacoat 603 and 5.0% Pharmacoat 904 to Fe_2O_3 was relatively high (9.9 and 9.6 cm, respectively) and the molecular lengths were short (33.2 and 47.0 nm, respectively). However, the IFD value in 6.5% Pharmacoat 603 was not very high (60.8) as compared with that in 5.0% Pharmacoat 904 (94.1), regardless of their similar properties. It might be considered that other factors such as the substituent content of methoxy and hydroxypropoxy groups are involved in the dispersibility of Fe_2O_3 particles[26].

Finally, the effects of adding polymers on the nanoparticulation of iron oxides were investigated (Table 3). Although the particle sizes of Fe_2O_3 and $Fe_2O_3{\cdot}H_2O$ were about 100-300 nm and 1-3 μm, respectively, according to the SEM micrographs (fig. 6a and b, respectively), the particles formed aggregates with sizes of 32.8 μm in Fe_2O_3 and 41.8 μm in $Fe_2O_3{\cdot}H_2O$ in distilled water.

After wet milling to disperse the iron oxides by Ultra Apex Mill with various polymers, the Fe_2O_3 particles milled with 0.75% or 0.90% HPC-H or 4.0% Pharmacoat 603 and $Fe_2O_3{\cdot}H_2O$ milled with 3.5% HPC-SSL did not reach nanometre size. Although the IFD value of Fe_2O_3 in the 0.45% and 0.5% HPC-H solutions was low (figs. 1 and 2 and Table 2), the particles were not dispersed to nanometre size even at the higher concentrations of HPC-H (0.75% and 0.90%). As mentioned earlier, adding polymers provides steric barriers to hinder reaggregation[16-19]. Therefore, it might be difficult for particles to decrease to a size that is less than the molecular length of the polymer added in a suspension, that is, the final particle sizes might depend on the size of each polymer because polymers with longer lengths (on the order of more than a micrometer) cannot make a sufficient coating layer on the nanoparticle surface owing to the larger polymer size. Yamamoto *et al.* reported on the stability of probucol nanoparticles using sodium dodecyl sulphate (SDS) and polyvinylpyrrolidone (PVP) K12 or K17 as dispersing agents, and they reported the importance of a sufficient coating layer of polymers for improved stability[27-30].

As it stands now, there are no effective ways to select an appropriate type and concentration of polymer to fabricate nanoparticles by wet milling, and it is a fact that various preexperimentations using various types and concentrations of polymers and surfactant are needed for nanoparticulation[13,15,31]. However, the IFD might provide a good way to indicate this. In Fe_2O_3, the IFD showed a relatively high value in the 6.5% Pharmacoat 603 solution (about 61, Table 2). Although the particle size did not reach into the nanometre range by wet milling with a lower concentration of Pharmacoat 603 (4.0%), the size was 0.75 μm in the 6.0% concentration (near the 6.5% concentration). In addition, the particles were in the nanometre size range with 5.0% and 6.5% Pharmacoat 904. The concentrations were around 5.5%, which provided a high IFD value (about 95, figs. 1 and 2). In $Fe_2O_3{\cdot}H_2O$, the IFD value was high in the 6.0% HPC-SSL solution (about 80, fig. 3). Use of 5.0% HPC-SSL (near the 6.0% concentration) achieved nanoparticulation (0.91 μm), but the use of 3.5% did not. When the IFD value of iron oxides is more than 60, nanomilling seems possible. By using the IFD value as a measure of nanoparticulation, cost, time and the wastage of active pharmaceutical ingredients could be avoided.

To develop stable medical inks of iron oxides, the dispersibility and nanoparticulation using various types and concentrations of polymers were investigated. The polymer concentration, surface tension and molecular length were found to affect the dispersibility of iron oxides and the solution viscosity. The optimal concentration of HPC-SSL, Pharmacoat 603, Pharmacoat 904 and Metolose SM-4, all of which have short molecular lengths, enabled successful fabrication of iron oxide nanoparticles by wet milling. In addition, we created an effective indicator for nanoparticulation, namely, IFD, for wet milling using Ultra Apex Mill. We believe that these methods and the information they provide would be useful in the pharmaceutical industry.

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