Separation and Characterization of Particles in Natural Water and Soil Using Flow Field-Flow Fractionation

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Abstract

Flow field-flow fractionation (FlFFF) is used to characterize particles in natural water (ground and surface water) and soil. The opposed flow sample concentration (OFSC) mode of FlFFF (OFSC-FlFFF) is employed, where the colloidal sample is continuously fed into the channel so that the particles are focused into a narrow band near the inlet of the FlFFF channel before the separation is initiated. There is no need for stopping the flow for the sample relaxation, which is usually required in conventional FlFFF operations. First, the OFSC-FlFFF is tested with mixtures of polystyrene latex spheres. Then the OFSC-FlFFF procedure is optimized for the analysis of particles in natural water and soil by varying various experimental parameters including the flow rates. Ground water of up to 100 mL has been successfully loaded, concentrated, and characterized by OFSC-FlFFF. Results show that the OFSC-FlFFF provides a simplified alternative to existing off-line concentration procedures, and it shows high potential for application to analysis of dilute colloidal particles in environmental water. The composition of the samples was analyzed using atomic absorption spectrometry.

Introduction

The particles in natural water play important roles in the fields of geology and environmental study (1–3). Both chemical and physical properties of the particles in natural water affect the transfer of toxic compounds (4,5) and, thus, need to be accurately determined in order to understand the transfer mechanism. Analysis of such environmental particles is not trivial, as they are usually complicated mixtures of particles having broad ranges in structure, mass, size, density, etc.

Various methods have been developed for the analysis of the physical and chemical properties of the natural particles (6). Still, accurate sizing of such complicated environmental particles remains a difficult task with no conventional methods proven to be satisfactory. One of the problems in sizing environmental particles is that they are usually in very low concentra-

tions and, thus, require pre-concentration. Ultra-filtration, centrifugation, and extraction have been used for pre-concentration of dilute environmental particles (7). Recently, cross-flow ultra-filtration has also been used for the concentration of marine colloids, surface water, and ground water (8). These off-line pre-concentration processes usually require extra steps, and may result in sample-loss or coagulation.

Flow field-flow fractionation (FlFFF) is a member of the field-flow fractionation family, which is applicable to separation and characterization of particulate and polymeric materials (9). It has been shown that FlFFF is applicable to analysis of various materials, including pharmaceutical (10), food (11), biological (12), and environment materials (13). For particulate samples, FlFFF provides separation of particles based on their hydrodynamic size, and thus allows the determination of particle size and its distribution. In an opposed flow sample-concentration (OFSC) mode of FlFFF (OFSC-FlFFF), a large amount of water sample can be loaded into the FlFFF channel, allowing on-line pre-concentration of the particles before the separation starts, thus eliminating the need for extra steps of off-line pre-concentration (14,15).

The aim of this study is to develop a method for the analysis of natural water (both ground and surface water) and the particles contained in the water or soil. In this study, OFSC-FlFFF, with a slight modification from previous studies, was tested and employed for the determination of the size and its distribution of the environmental particles in ground or surface natural water and in soil. Atomic absorption spectrometry (AAS) was also used for the determination of their chemical compositions (mainly the metal content).

Theory

In FlFFF, for well-retained particles, the retention time (t_r) is related to the hydrodynamic diameter (d_H) in the literature (16).

$$t_r = \frac{}{2kT} \frac{}{V} d_H$$

Eq. 1

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where w is the channel thickness, $?$ is the carrier viscosity, V_c is the cross flow rate, k is the Boltzmann constant, T is the absolute temperature, and V is the channel flow rate. Equation 1 indicates that the retention time (t_R) is directly proportional to the hydrodynamic diameter (d_H). Using equation 1, a FlFFF fractogram can be converted to a size distribution. The full expression of equation 1 is rather complicated, and has been shown in earlier publications (9–16). In this work, the conversion of the FlFFF fractogram to the size distribution was made using the full expression with the laboratory software.

Experimental

Sampling

Four samples were collected at two distant regions of the city of Gwang-Ju (Korea). From region 1, a ground water ("GW-1") and a surface water ("SW") sample were collected. The SW sample was colleted at about 1 m from the edge of the Gwang-Ju stream and below the stream surface, which is less than 500 m apart from the sampling site of the GW-1. From region 2, a ground water ("GW-2") and a soil ("S-1") sample were collected. The sampling site of the GW-2 was about 150 m apart from that of the "S-1" sample. Both GW-1 and GW-2 are emergency drinking sources. All water samples (GW-1, GW-2, and SW) were filtered through a 1.2-µm membrane filter (Anodisc 47, Whatman, Florham Park, NJ) at the sampling site to remove large particles. The "S-1" sample was collected at the depth of 1 m and was transported to the laboratory, where the sample was dispersed in water containing 0.1% FL-70 by 1 min-sonication, and then passed through a filter (1.2 µm Millipore GF/C, Bedford, MA) to remove large particles. In order to maintain the uniform colloidal state, all samples were stirred for 30 min in a mass cylinder at room temperature before being analyzed by FlFFF.

AAS

A Perkin Elmer Model 3100 AAS (Norwalk, CT) was used for elemental analysis of the samples. For analysis of Hg and As, a Perkin Elmer Model 5100 AAS equipped with a Perkin Elmer FIAS 400 Hydride Generation system was used. For analysis of water samples (GW-1, GW-2, and SW), 8 metal elements (Fe, Mn, Cu, Pb, Zn, Ni, Cr, and Cd) were determined according to the EPA protocol 3010A (17) and 7000A (18), where 100 mL of sample was acidified with 1 mL of c-HNO_3, and then analyzed by a flame-AAS. For determination of As, 25 mL of sample was treated for reduction with 5 mL of 5% KI and 5 mL of 5% vitamin C and was diluted to 50 mL with pure water, and then analyzed by flow injection analysis system (FIAS)-AAS, where water containing 0.2% $NaBH_4$ (as a reducing agent) and 10% HCl was used as the carrier solution. A cold vapor method was used for analysis of Hg, where 50 mL of sample was pretreated and determined by EPA 7470 protocol. As for the soil sample, approximately 0.5 g of sample was wetted with 10 mL of water in a beaker, and then 20 mL of aqua regia was added. The beaker was heated on a hot plate, and then dried. c-HCl (5 mL) was added, and then, after cooling, the solution was diluted with pure water, transferred into 100-mL volumetric flask, and then analyzed.

FlFFF

A FlFFF system was assembled in a similar manner as reported previously (19). The membrane used for the accumulation wall was a regenerated cellulose membrane (YM10, Amicon, Inc., Houston, TX) having the cut-off molecular weight of 10,000 Dalton. The thickness of the Mylar spacer was 194 µm. The void volume of the channel was measured to be 1.07 mL from the retention time of the void peak of the sample. The detector was a UV detector (M720, Young Lin, Korea) set at 254 nm. The carrier was pure water containing 0.1% sodium dodecyl sulfate (SDS) and 0.02% sodium azide (both from Sigma, St. Louis, MO). The particle standards were polystyrene latex beads having nominal diameters of 28, 79, and 138 nm obtained from Duke Scientific Corp. (Palo Alto, CA).

The operation of FlFFF in the OFSC mode is shown in Figure 1. Three HPLC pumps (M930, Young Lin, Korea) were used to provide the channel-flow, opposed flow, and the cross-flow, respectively, and one peristaltic pump (Miniplus 3, Gilson, France) was used for loading the channel with the sample. OFSC-FlFFF was performed in four steps: (*i*) sample-loading, (*ii*) focusing, (*iii*) relaxation, and finally (*iv*) elution. At the first step (sample-loading step), the 3-way valves 1 and 3 were set so that both the channel-flow pump and the peristaltic pump provided the flows going into the channel from both ends of the channel. The sample was fed into the channel by a peristaltic pump through the channel outlet. In the sample-loading step, the cross-flow was maintained at low flow rate (~ 0.2 mL/min) to keep the particles away from the upper wall. After the sample-loading was complete, the peristaltic pump was turned off and valve 3 was switched so that the focusing pump (instead of the peristaltic pump) provided the flow going into the channel through the channel outlet (focusing step). At this step, the sample was focused into a narrow band near the inlet of the channel. The position of the initial sample band was determined by the flow rate ratio of the channel flow–focusing flow (20). Next, valve 1 and 3 were set to bypass, and valve 2 was opened so that only the cross-flow pump provided the flow going into the channel to relax the sample (relaxation step). During the relaxation step, the channel-flow was set at the desired level for the elution of the sample. After the sample was fully relaxed, valves 1 and 3 were switched back to their normal elution positions for the sample elution (elution
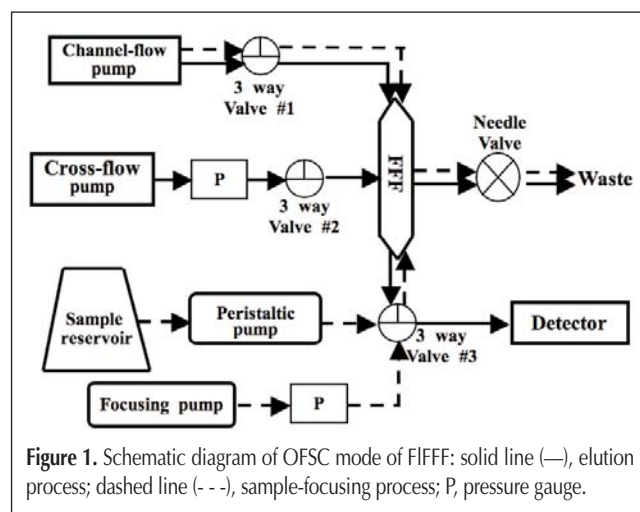

Figure 1. Schematic diagram of OFSC mode of FlFFF: solid line (—), elution process; dashed line (- - -), sample-focusing process; P, pressure gauge.

step). The OFSC mode of FlFFF (OFSC-FlFFF) allowed concentration of the particles by loading a large amount of the sample.

Results and Discussion

Polystyrene latex separation by FlFFF

Figure 2 shows fractograms of a mixture of three polystyrene (PS) latex beads having nominal diameters of 28, 79, and 138 nm obtained by OFSC-FlFFF at various focusing times. The total sample-loading volume was 50 mL. During the focusing step, the incoming flow rates through the inlet (channel-flow) and the outlet (focusing flow) of the channel were 0.2 and 3 mL/min, respectively, with the flow rate ratio of 1:15. For the sample elu-
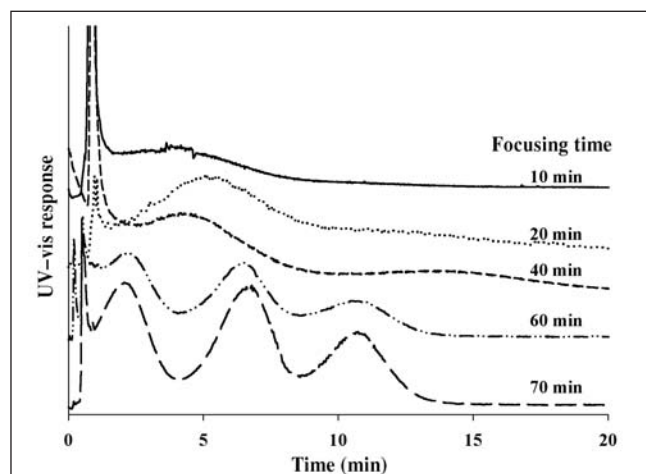
Figure 2. Separations of polystyrene latex beads (28, 79, and 138 nm) obtained by OFSC-FlFFF with various focusing times. The sample-loading volume was 50 mL. During the focusing step, the incoming flow rates through the inlet and the outlet of the channel were 0.2 and 3 mL/min, respectively, (flow rate ratio = 1:15). For the sample elution, the cross-flow (V_c) and the channel-flow (V) were 0.8 and 2.0 mL/min, respectively.
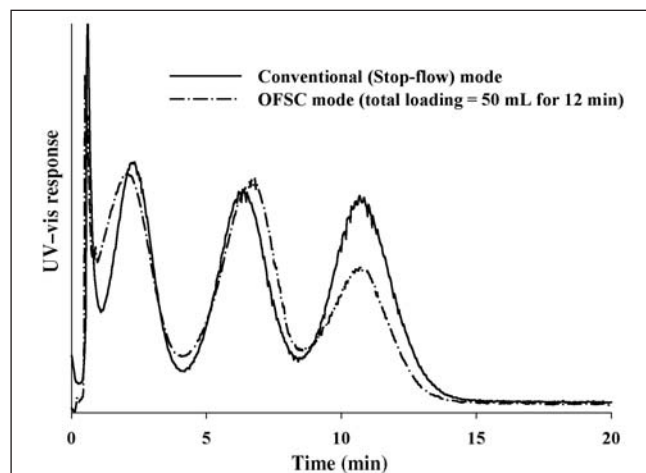
tion, the cross-flow (V_c) and the channel-flow (V) were 0.8 and 2.0 mL/min, respectively. As shown in Figure 2, as the focusing time increases, the resolution gradually increases, and, finally, at the focusing time of 70 min, a good separation was obtained. It seems that when a focusing time lower than 70 min is used, the particles are not fully focused into a narrow band.

Figure 3 shows two fractograms of the same mixture of PS beads obtained by conventional stop-flow (solid line) and OFSC mode (dotted line) of FlFFF. In the stop-flow mode, the mixture colloid was injected using a 20-μL loop injector. In the OFSC mode, the same amount (20 μL) of the mixture was diluted with the carrier liquid to 50 mL and then loaded into the channel. Total loading volume was 50 mL, and the focusing time was 70 min. All other OFSC-FlFFF experimental conditions were the same as those in Figure 2. As shown in Figure 3, no significant differences were found between the results from the OFSC and conventional stop-flow mode of FlFFF, except for 138 nm PS. For 138 nm particles, the peak area obtained by OFSC-FlFFF is smaller than that by the stop-flow mode, probably due to adsorption of the particles onto the membrane during the long focusing step (70 min). Nevertheless, it seems that the OFSC method is applicable to particles smaller than approximately 100 nm.

The diameters of the PS beads determined by the conventional and the OFSC modes of FlFFF are summarized in Table I. The diameters determined by the two different modes of FlFFF are in good agreement. The results shown in Figure 3 and in Table I suggest the OFSC-FlFFF can be useful for on-line concentration and size-based separation of particles in natural water, where the

Table I. Diameters of Polystyrene Latex Standards Measured by Two Different Modes of FlFFF

Nominal diameter (nm)	Diameter determined by FlFFF (nm)	
	Stop-flow mode	OFSC mode
28	26.2	25.7
79	78.9	80.7
138	138.4	139.2

Figure 3. Separations of polystyrene latex beads (28, 79, and 138 nm) obtained by stop-flow (solid line) and OFSC mode (dotted line) of FlFFF. In OFSC mode, the total loading volume was 50 mL, and the focusing time was 70 min. All other experimental conditions were same as those in Figure 2. In the stop-flow mode, the sample was injected with a 20-μL loop injector.
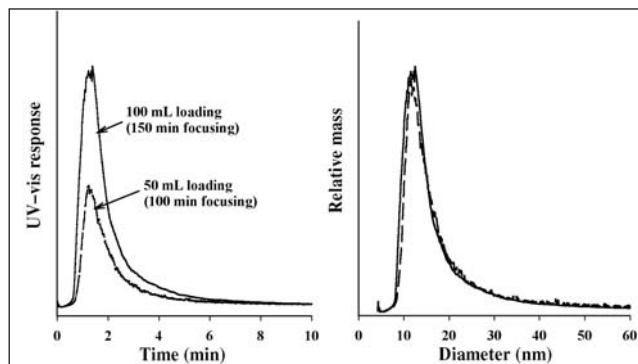
Figure 4. Fractograms and size distributions of the particles in GW-1 ground water obtained by OFSC-FlFFF with the total sample loading of 100 and 50 mL. For the elution of the sample, the cross-flow (V_c) and the channel-flow (V) were 0.4 and 1.0 mL/min, respectively. All other conditions are the same as those in Figure 2.

particle concentration is usually low.

OFSC-FlFFF analysis of natural particles

Figure 4 shows fractograms and size distributions of the particles in GW-1 ground water obtained by OFSC-FlFFF with the total sample-loading of 50 and 100 mL. At the elution step, the cross-flow (V_c) and the channel-flow (V) were 0.5 and 1.0 mL/min, respectively. All other conditions were the same as those in Figure 2. The focusing time was 100 and 150 min for the sample-loading of 50 and 100 mL, respectively. When the sample loading was increased by two times, the peak area was also increased by almost two times (2.12×10^7 vs 1.07×10^7). Despite the difference in the sample-loading volume, the measured size distributions agree well. This result suggests that OFSC-FlFFF can be used not only for the size-based separation (thus, for the determination of the size distribution), but also for determination of the relative amount of the particles in natural water.

Figure 5 shows fractograms of all four samples obtained by OFSC-FlFFF, with the total sample loading of 50 mL, and Figure 6 shows the size distributions determined from the fractograms shown in Figure 5. For the elution of the sample, the cross-flow
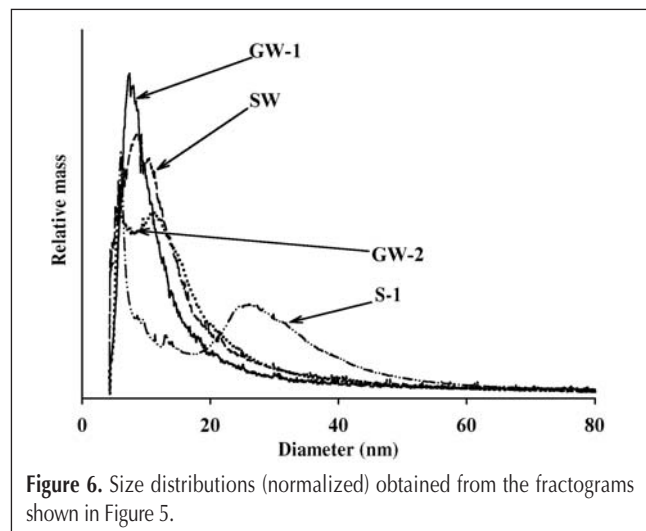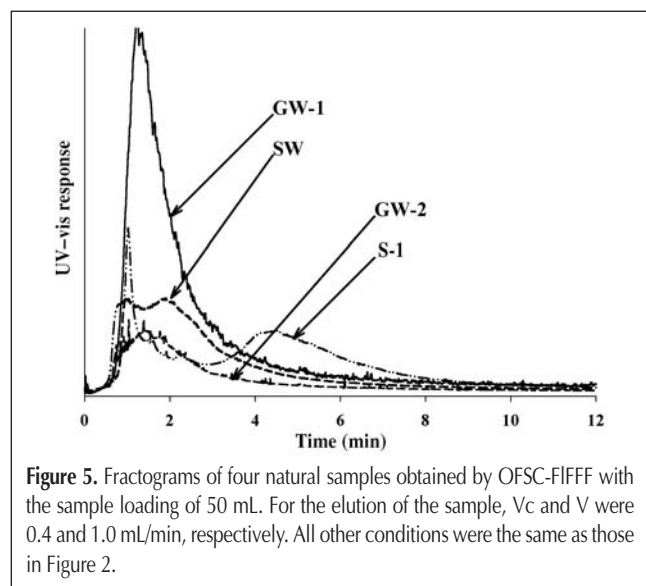
(V_c) and the channel-flow (V) were 0.4 and 1.0 mL/min, respectively. All other conditions were the same as those in Figure 2. It is interesting to see that the size distributions of the particles in GW-1 and SW are somewhat similar, perhaps because the sampling sites of GW-1 and SW were in the same area of the city of Gwang-Ju (less than 500 m apart). The peak area of the GW-1 is, however, much higher than that of the SW, suggesting the particle content is much higher in GW-1 than in the SW.

On the other hand, the size distribution of the particles in GW-2 is quite different from that of the S-1 sample, although the sampling sites of both GW-2 and S-1 sample were close to each other (about 150 m apart). This may suggest that there is a low probability that the particles in GW-2 are related to the S-1 soil particles. Also, the peak area (thus the particle content) and the size distribution of the GW-1 appear to be somewhat different from those of GW-2.

Analysis of chemical composition by AAS

Results obtained by AAS for all samples are summarized in Table II. All water samples (GW-1, GW-2, and SW) were filtered

Figure 5. Fractograms of four natural samples obtained by OFSC-FlFFF with the sample loading of 50 mL. For the elution of the sample, Vc and V were 0.4 and 1.0 mL/min, respectively. All other conditions were the same as those in Figure 2.

Figure 6. Size distributions (normalized) obtained from the fractograms shown in Figure 5.

Table II. Concentration of Elements in Natural Samples

	Concentration in					
Element	Filtered GW-1* (mg/L)	Filtered GW-2* (mg/L)	Filtered SW* (mg/L)	GW-1 particle[†] (mg/kg)	GW-2 particle[†] (mg/kg)	S-1[†] (mg/kg)
Fe	0.22	23.4	0.91	426,000	523,000	35,700
Mn	0.03	1.70	0.23	510	440	503
Cu	0.01	< 0.01	0.18	270	46	13
Pb	< 0.01	< 0.01	< 0.01	1,540	20	35
Zn	0.30	0.31	0.12	10,300	5,250	51
Ni	< 0.01	0.03	< 0.01	106	39	50
Cr	< 0.01	0.13	< 0.01	1,420	657	181
Cd	< 0.01	0.01	< 0.01	< 0.01	< 0.01	4.0
As	0.002	< 0.001	0.001	< 0.05	< 0.05	< 0.05
Hg	< 0.001	< 0.001	< 0.001	< 0.05	< 0.05	< 0.05

* After filtered through a 1.2 μm filter.
[†] Collected on a 1.2 μm filter.

Figure 7. Elemental compositions of GW-1 and SW.
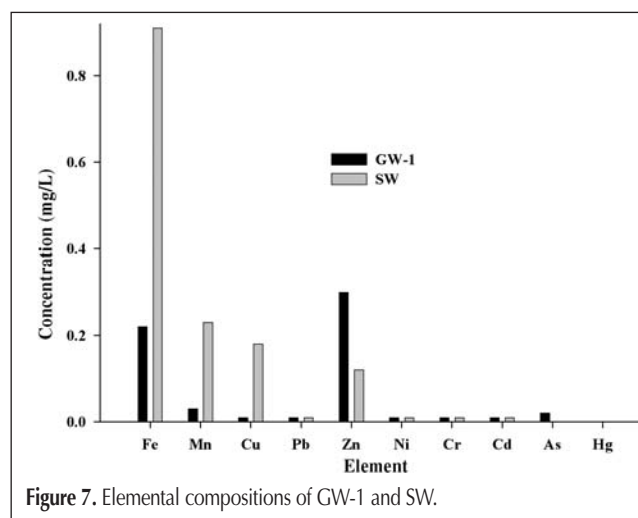
through a 1.2-µm filter, and then the filtered water and the particles collected on the filter paper were analyzed by AAS, respectively (see the Experimental section).

Both the filtered GW-1 and the SW water samples contain the same kinds of elements as shown in Table II and in Figure 7. Having similarities in both the size distributions (Figure 6) and the elemental compositions may indicate the GW-1 and the SW are related to each other in one way or another, although a more detailed investigation is required for complete understanding of the inter-relationship between closely located natural waters. The concentrations of the elements are much higher in the filtered SW than in the filtered GW-1, except Zn, which has a concentration higher in filtered GW-1 than in the SW. Generally the concentrations of the elements in ground water are lower than those in surface water due to the "self-purification" of the ground water in the process of migration (e.g., filtration through the soil) (21). Higher Zn concentration in the GW-1 than in the SW is probably due to oxidation of the galvanized Zn on the inner wall of the steel pipe that was used to pump the GW-1 ground water (22). It is noted that the concentrations of Fe and Zn are much higher than those of the other elements in the particles of the GW-1 sample ("GW-1 particle").

No particular trends were observed between another pair (GW-2 and S-1 samples) of samples whose sampling sites were close to each other.

Conclusion

OFSC mode of FlFFF has been employed successfully for pre-concentration and size-based separation of the colloidal particles in natural water, which yields information on the relative amount and the size distribution of the particles. Results show the OFSC-FlFFF is useful for the analysis of particles in natural water whose particle content is extremely low and pre-concentration is required. OFSC-FlFFF provides a simplified alternative to existing off-line pre-concentration procedures and shows high potential for application to analysis of dilute colloidal particles in environmental water.

Use of OFSC-FlFFF and AAS provides useful information on contamination of natural water and could become a useful tool for environmental studies for understanding of transfer mechanisms of toxic compounds or for tracing of the contamination source of ground water.

Acknowledgments

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