Analysis of Red Blood Cell Movement in Whole Blood Exposed to DC and ELF Electric Fields

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To evaluate hematological effects of direct current (DC) and alternating current (AC) extremely low frequency (ELF) electric field exposure, this study investigated red blood cell (RBC) movement in whole blood. Video images of RBCs were recorded under a microscope using specially designed electrode systems. Video analysis software was then used to measure the RBC velocity. The noise level and measurement system stability were confirmed based on results of a no-field exposure experiment. Using the electrode system to produce a non-homogeneous electric field, different movements were found to occur in DC and AC field exposure. The RBCs moved in the directions of the electric field and the gradient of field distribution, respectively, in the DC and AC fields. Dependences of the RBC velocity on the field strength were, respectively, linear and quadratic in the DC and AC fields. These results suggest that electrophoretic and dielectrophoretic movements were, respectively, dominant in the DC and AC fields. The magnitude of the electric field necessary to cause these effects was found to be $10^3$–$10^5$ times greater than the internationally publicized guideline for human safety. Bioelectromagnetics. 43:149–159, 2022. © 2022 Bioelectromagnetics Society.

Keywords: dielectrophoresis; electrophoresis; ELF electric field; erythrocyte; whole blood

INTRODUCTION

The electrification of daily life has progressed remarkably. Today, we are surrounded by numerous electrical devices. Various high-powered electrical devices have come to be used in our daily living environment and in medical settings. Since 1979, when effects of commercial frequency electromagnetic fields on a human body were reported, many studies have been conducted [Wertheimer and Leeper, 1979; National Research Council US, 1997; World Health Organization, 2007; ICNIRP, 2010]. Based on those results, international organizations have provided guidelines for biological safety [World Health Organization, 2007; ICNIRP, 2010]. Each country has established safety standards for its residents [Polk, 2017]. As biological effects of electromagnetic fields, the thermal effects of high-frequency electromagnetic fields and the body surface stimulating effects of low-frequency electromagnetic fields have been identified [Odagiri et al., 1994; Shimizu and Shimizu, 1999]. This study specifically examines some biological effects of direct current (DC) and alternating current (AC) electric fields inside a human body, for which the mechanisms remain unclear. The frequency range of the DC electric field used for this study is extremely low frequency (ELF, 0–300 Hz). Because ELF includes commercial power frequencies such as 50 and 60 Hz, various studies have...
elucidated their biological effects [Carstensen, 1987; Reilly, 1992, 1998; Wilson et al., 1990].

Among studies of the biological effects of ELF electromagnetic fields, many have assessed hematological effects [Wilson et al., 1990; Takebe et al., 1999; World Health Organization, 2007]. When blood is exposed to an electric field, various physical changes occur. For example, the pearl chain effect, by which blood cells are chained when exposed to a high-frequency electric field, is well known. From physiological chemistry analyses, blood cells are known to move in a liquid by electrophoresis or dielectrophoresis [Cruz and Garcia-Diego, 1998; Hsu et al., 2003; Lu et al., 2003; Minerick et al., 2003; Leonard and Minerick, 2011; Su et al., 2012]. For example, experiments have been reported in which red blood cells (RBCs) in a plastic capillary tube are spatially separated by dielectrophoresis and are thereby classified into ABO-Rh types [Srivastava et al., 2011]. Nevertheless, it is usually difficult to observe these phenomena in whole blood because of high blood cell density and high blood viscosity. Therefore, in most of these reports, blood diluted with saline solution has been used to facilitate cell migration and its observation. Nevertheless, no report of the relevant literature describes that such a phenomenon occurs inside the body when an ELF electric field is applied outside the body. Electric field exposure from outside the body induces an electric field inside the body. One might infer that the movement of blood cells, which are charged particles, is affected by such an induced electric field. For this study, we assessed direct field exposure to elucidate the electric field effects on whole blood. Whole blood includes many blood components, but we specifically examined the movement of RBCs, which make up more than 90% of all blood cells. Although we were unable to simulate the situation inside the body completely, the main purpose of this study is to investigate the possibility of electric field effects on whole blood in the well-controlled measurement in vitro. If the possibility were verified, then it would provide strong motivation for future in-vivo studies.

MOVEMENT OF RBCs IN AN ELECTRIC FIELD

**Electric Field Exposure to Human Body**

Figure 1 shows a human body modeled in a vertical uniform electric field on the ground under a high-voltage power transmission line. The electric field concentrates on the top of the head. The electric field inside the body is expressed as [Kaune and Forsythe, 1985; Chen et al., 1999]

\[
E_i \approx \frac{\omega \varepsilon_0}{g_i} f_{e} E_o
\]

where \(E_i\), \(E_o\), \(\omega\), \(\varepsilon_o\) \(g_i\), and \(f_e\), respectively represent the internal electric field intensity, external electric field intensity, angular frequency of the electric field, permittivity of the external medium, conductivity of the internal medium, and an electric field enhancement factor related to body shape. For a human body in air, the internal electric field is usually greatly attenuated because the internal conductivity is greater than the external permittivity. However, when the external electric field intensity is high or when the field enhancement factor is large, the internal electric field cannot be ignored.

The International Commission on Non-Ionizing Radiation Protection (ICNIRP) has established guidelines for human health protection based on evaluation results from the World Health Organization (WHO). In the guidelines, the electric field intensity in the body is used as an evaluation index of the basic limit for low-frequency radiation below 100 kHz [ICNIRP, 2010]. For this study, we applied an electric field to whole blood and analyzed RBC movement to investigate blood cell movement caused by DC and ELF AC electric fields.

**Forces Associated With Electric Field Exposure**

One biological effect of electric fields in the body is the mechanical effect on charged particles. The body holds various charged particles. We specifically examined RBCs because of their large amount in the body and because of their physiologically important roles. Actually, RBC surfaces are known to be negatively charged under normal conditions [Jan and Shu, 1973]: RBCs move by receiving electric force in the direction opposite to the electric field direction. Therefore, electric...
field exposure from outside the body might affect RBC movement in the body.

During electric field exposure, although various physical forces act on RBCs in blood, they are mainly electrophoresis, dielectrophoresis, electro-osmosis forces, and gravity. The following equation incorporating their respective mobilities shows the velocity of a charged particle subjected to these forces in a liquid [Srivastava et al., 2011].

$$\vec{v} = \mu_{EP}\vec{E} + \mu_{DEP}\nabla E^2 + \mu_{EO}\vec{E} + \vec{v}_g,$$  \hspace{1cm} (2)

where

$$\mu_{EP} = \frac{Q}{6\pi r \eta},$$  \hspace{1cm} (3)

$$\mu_{DEP} = -\frac{\pi r^3 f_{CM} \varepsilon_m}{3 \eta},$$  \hspace{1cm} (4)

$$\mu_{EO} = \frac{\varepsilon_m \zeta}{\eta},$$  \hspace{1cm} (5)

$$\vec{v}_g = \frac{2r^2}{9\eta}(\rho_p - \rho_L)g\sin\theta.$$  \hspace{1cm} (6)

Therein, \(v\), \(E\), \(\mu_{EP}\), \(\mu_{DEP}\), \(\mu_{EO}\), \(v_g\), \(r\), \(f_{CM}\), \(\varepsilon_m\), \(\eta\), \(Q\), \(\zeta\), \(\rho_p\), \(\rho_L\), and \(g\), respectively denote the RBCs final velocity, electric field intensity at RBCs, electrophoretic mobility, dielectrophoretic mobility, electroosmotic mobility, velocity caused by gravity (equivalent) particle radius, the Clausius–Mossotti coefficient, medium permittivity, medium viscosity, particle surface charge, particle zeta potential, particle density, medium fluid density, and acceleration of gravity. Arrows over variables signify vectors.

**Effects on RBCs in the Body**

Blood vessels inside the human body run in all directions, and effects of gravity are largely canceled out. Therefore, for this study, we consider the first three terms in Equation (2), which represent the main effects of electric field exposure.

Both the first and third terms are proportional to the electric field intensity. Using general blood parameters (\(Q = -3.19 \times 10^{-10} \text{ C}\), \(r = 2.70 \times 10^{-6} \text{ m}\), \(\eta = 1.58 \times 10^{-3} \text{ Pa s}\), \(\varepsilon_m = 15.7 \times 10^{-3} \text{ V}^{-1} \text{ m}^{-1}\)) [Gudmundsson and Bjelle, 1993; Gutsul et al., 2012; Tokumasu et al., 2012; Zhbanov and Yang, 2015], the ratio of \(\mu_{EP}\) to \(\mu_{EO}\) is estimated as on the order of 10^6. In other words, the mobility of electrophoresis is much greater than that of electroosmosis. From these findings, we chose to investigate electrophoresis and dielectrophoresis as electric field exposure effects on RBC movement.

Equation (2) is valid irrespective of whether the electric field is DC or AC. The equation shows that the particle motion direction is that of the electric field vector in electrophoresis. The particle motion direction is that of the gradient of the spatial distribution of the squared electric field in dielectrophoresis. Because of the high conductivity prevailing in a living body, extremely large gradients in electric field are unlikely. Under general conditions such as the blood parameters presented above, electrophoresis is far larger than dielectrophoresis. Therefore, electrophoresis is regarded as dominant during DC electric field exposure. This is also evident from experimentally obtained results presented in the following sections. However, for AC, the electric field vector direction reverses over time. When the particle movement cannot follow the frequency change of the electric field, similarly to RBCs in whole blood, the electrophoretic effect is canceled out. These considerations suggest that electrophoretic mobility is dominant during exposure to DC electric fields, whereas dielectrophoretic mobility is dominant during exposure to AC electric fields.

**MEASUREMENT OF RBC MOVEMENT IN DC ELECTRIC FIELD EXPOSURE**

As described above, the movement of electrophoresis is proportional to the electric field vector. Therefore, negatively charged RBCs receive the force and move in the direction opposite to the electric field. They reach a constant velocity in the balance with resistance because of the blood plasma viscosity. The final velocity is presumed to be proportional to the electric field intensity. The following experiment was conducted to analyze this electrophoresis phenomenon of RBCs. In the experiment, the RBC movement associated with the application of a DC electric field was observed under a microscope and was recorded as a video image. Then video image analysis was applied. The video frame interval was set as 20 fps.

A schematic diagram of the experiment sample is presented in Figure 2. A copper foil with 0.02 mm thickness was pattern-etched on a translucent glass epoxy plate with 1.6 mm thickness to form parallel electrodes. The inter-electrode distance was 5.0 mm. A blood sample of a certain thickness was prepared by dropping the whole blood between the electrodes without diluting it, and then covering it with a cover glass. The sample thickness was 0.02 mm, which is of
the same order as the blood capillary diameter, but it differs from capillaries because of the horizontal spread between the electrodes on the slide glass. Comparison of the conditions in vivo reveals that there was neither blood pressure nor pulsatile flow. The substrate was a light-transmitting glass epoxy. Blood cells were visible when using a normal transmission microscope. Blood samples were taken from the tip of the ring finger of the left hand of a 27-year-old healthy woman by her own self-puncture after she gave informed consent. No anticoagulant was used in the blood sample. This experiment was conducted at laboratory temperatures of 22–26 °C. The sample temperature was monitored during the experiment using a thermography camera (FLIR C2; FLIR Systems, Wilsonville, OR). The average temperature over the observation area in the measurement period was 26.8 °C. The slope of the linear regression curve for the temporal change of the sample temperature over 90 s was close to zero (<0.003); the coefficient of variation was less than 0.015. This finding indicated that the sample temperature remained constant during the measurement period, and indicated that no heat generation in samples occurred because of the electric field exposure.

Figure 3 presents one typical frame of the video image observed under the microscope. Unlike conventional experiments using diluted blood, whole blood was used for this study. Therefore, RBCs are observed as overlapping, but blood cells are clearly recognizable. The individual blood cell movement can be measured. For blood cell motion analysis, the electric field direction between the parallel electrodes was designated as the X-axis. The orthogonal direction was designated as the Y-axis.

To examine the uniformity of the electric field along the X-axis, the voltage drop with respect to the distance from an electrode was measured in the whole blood sample. The drop was linear with respect to distance. This finding demonstrates that the electric field remains constant irrespective to the X-coordinate. The RBCs apparently experience the same electric field exposure along the X-axis. Results of these experiments confirmed that the RBC velocity remained constant along the X-axis, except in the proximity of an electrode. The maximum deviation from a linear regression curve was less than 17%. For the following analysis, the velocity was measured at the center of the 5 mm distance between the electrodes.

For image analysis, MATLAB [2018] was used to measure frame-to-frame changes in the RBC positions. After tracing the motions of 10 arbitrarily chosen RBCs for the experiment, their mean velocities and standard deviations were calculated.

First, as control data, we measured the RBC motion with no field exposure. Figure 4 shows the change over time of the mean ± standard deviation of the RBCs’ velocity. The horizontal axis is the elapsed time after the start of observation. The vertical axis is the blood cell velocity as calculated from the distance of a chosen blood cell between frames of the video image. The same applies to all waveforms of temporal changes in subsequent figures. Without an electric field, no remarkable movement was observed other than random fibrillation of blood cells. From this result, low noise and stability of this measurement were confirmed.

Next, as presented in Figure 2, DC voltage was applied between parallel electrodes to measure blood cell movement. A two-pole twin-throw switch was installed between the power supply and the electrodes so that the direction of the electric field was switchable instantly between left and right. The experimentally obtained results are presented in Figure 5. When +10 V was applied, the electric field was in the −X direction. The negatively charged RBCs moved in the +X direction. When −10 V was applied, all directions were opposite. The blood cell velocity changed markedly in the opposite direction to the electric field during exposure to the electric field.

The blood cell velocity tends to decrease with time even with constant voltage. When we diluted whole
blood with saline, the velocity decay was suppressed. The degree of the suppression corresponded to the dilution ratio. No noteworthy change around the electrode–fluid interface was observed, except for some RBC clustering. This finding suggests that the major cause of this velocity decay can be the high viscosity of the whole blood. Additionally, the velocity decay might be attributable to nonlinearity of the charge state in the viscous fluid mixture consisting of blood cells and plasma. Nevertheless, details of this phenomenon remain as issues for future study.

To verify that the electric field truly caused this phenomenon, we conducted the same experiment by changing only the electric field intensity while maintaining the other conditions as constant. The experimentally obtained results are depicted in

![Fig. 4. Temporal change of RBC velocity in no electric field exposure, with X and Y components of velocity vector. Error bars show mean ± standard deviation for N = 10, as all figures hereafter. RBC = red blood cell.](image)

![Fig. 5. Temporal change of RBC velocity with DC electric field exposure: RBCs with negative charge move in opposite direction to electric field. DC = direct current; RBC = red blood cell.](image)
We observed blood cell movements with electrode spacing of 5.0 mm and inter-electrode voltage as small as 2.0 V, i.e. an electric field of 0.40 kV/m. As depicted in Figure 6, the blood cell velocity in the electric field direction changed as the electric field intensity increased or decreased. In Figure 6, even with the same inter-electrode voltage, the velocity during the latter half of the measurement (after 45 s) tends to be lower than in the first half. This phenomenon is also apparent in the case of other long-term measurements without application of the electric field. For example, the effects of stable gravity were examined by inclining the microscope. A slight decrease in RBC velocity was observed as time progressed. From these facts, that decrease can be regarded as a result of a slight increase in the viscosity of the whole blood sample without using anticoagulant. In Figure 6, white noise-like movement was also apparent in the direction orthogonal to the electric field. This movement probably reflects the irregular movement which occurs when flat disk-shaped RBCs move in the viscous blood plasma.

To elucidate the relation between the exposed electric field intensity and the blood cell velocity, the average blood cell velocity during the exposure period was calculated. Figure 7 shows the relation between exposed field and the blood cell velocity, i.e. dose–response. Because the distance between the electrodes was constant, the exposed electric field intensity on the horizontal axis was proportional to the applied voltage. Results show that the DC electric field causes blood cells to move through the plasma in the field direction, and that the velocity of movement is proportional to the electric field intensity. These findings suggest that the blood cell movement during DC electric field exposure is an electrophoretic phenomenon.

**MECHANISMS OF RBC MOVEMENT**

We investigated the RBC movement mechanisms associated with electric field exposure described in the preceding section. From the discussion presented in the third part of section “MOVEMENT OF RBCs IN AN ELECTRIC FIELD,” electrophoresis and dielectrophoresis were postulated to be major causes of blood cell movement when exposed to an electric field. The velocity of RBCs is proportional to the electric field intensity in the former case and to the gradient of the squared electric field intensity in the latter case. For parallel electrodes presented in Figure 2, the electric field is distributed almost uniformly in the observation area. No dielectrophoretic force can be expected. To
elucidate this point, we applied the AC electric field with parallel electrodes and observed the RBC velocity. The result closely resembled that portrayed in Figure 4, or indicated no apparent velocity change. This constitutes evidence for the cell movement explained in the third part of the second section “MOVEMENT OF RBCs IN AN ELECTRIC FIELD,” Effects on RBCs in the body). Therefore, we newly developed the electrode structure presented in Figure 8.

Because the distances between the left and right electrodes differ depending on the vertical position of the electrodes in Figure 8, a non-uniform electric field distribution is generated in the center of the observation area. In other words, on the symmetry axis of the left and right electrodes, the electric field becomes stronger toward the top. A gradient of the electric field intensity is generated in the vertical direction of Figure 8.

We manufactured such electrodes and conducted an experiment to assess the blood cell movement mechanisms. The symmetrically inclined non-parallel electrodes were prepared by etching copper foil on a glass epoxy circuit board. The electrodes were 0.02-mm-thick 25 mm × 10 mm rectangles with 45° inclination and spacing of 1.0 mm at the top and 28 mm at the bottom. The spatial distribution of the electric field between these electrodes is not uniform. In fact, the magnitude of the electric field changes corresponding to $y^{-1}$ along the $Y$-axis. The gradient of squared electric field changes corresponding to $y^{-3}$. The velocity change along the $Y$-axis was confirmed from experiments. To avoid the mechanical effects of electrodes on the RBC movement, the cell velocity was observed at the center (origin of $X$–$Y$ coordinate) area of the cover glass. Because of the left-right symmetry, the direction of the electric field on the symmetry axis is horizontal, or in the $X$ direction.

For the electrode structure presented in Figure 8, both electrophoresis and dielectrophoresis can occur. Therefore, we first investigated the possibility of both in a DC electric field. Figure 9 portrays the RBC velocity on the symmetry axis when a DC voltage is applied to these electrodes. As in the case of parallel electrodes in Figure 2, the RBCs moved in a horizontal direction, i.e. in the electric field direction. This finding is consistent with an earlier prediction described in section “MOVEMENT OF RBCs IN AN ELECTRIC FIELD”: when electrophoretic force and dielectrophoretic forces work simultaneously, the strength of the former is orders of magnitude greater than that of the latter. In Figure 9, some movement is apparent also in the $Y$ direction, although it is slight.
compared to that in the X direction. This fact might be attributable to irregular movement of RBCs in the viscous fluid, such as rotations in migration.

Figure 10 presents results obtained when an AC electric field of 50 Hz was applied to the same electrodes. In contrast to the case of the DC electric field, the RBCs moved not in the horizontal direction but in the vertical direction, i.e. in the direction of the gradient of the electric field intensity. The reason for this finding is regarded to be that explained below.

Blood is a non-Newtonian viscous fluid, with viscosity of $3-4 \times 10^{-3} \text{ Pa} \cdot \text{s}$ under normal conditions. In the experiment using whole blood of a healthy subject, the RBC velocity in the 20 µm thickness slab sample was on the order of 1 µm/s. In the 50 Hz AC electric field, the horizontal direction of the velocity is altered every 20 ms. The RBCs move only 0.02 µm distance during this period. This distance was too small to detect in our observation system. Therefore, the RBC electrophoretic movement in the horizontal
direction in proportion to the electric field could not
be observed. However, the dielectrophoretic velocity
is proportional to the gradient of the squared electric
field. It, therefore, becomes the major factor affecting
RBC movement. These analyses of experiments
clarified electrophoresis as the main mechanism of
blood cell movement during exposure to a DC electric
field. Dielectrophoresis is the mechanism of blood cell
movement which occurs during exposure to an AC
electric field.

ANALYSIS OF BLOOD CELL MOVEMENT IN AN AC
ELECTRIC FIELD

Blood cell movements during exposure to the 50 Hz
AC electric field were investigated further through
experimentation, assuming that the human body was
exposed to the ELF electric field. For the experiment, the
electrodes presented in Figure 8 were used. The blood cell
movement was analyzed from video images taken under a
microscope using the method described earlier in section
"MEASUREMENT OF RBC MOVEMENT IN DC ELECTRIC FIELD EXPOSURE."

Figure 11 portrays the temporal change of the
measured blood cell velocity with different applied
voltages. The degree of change in RBC velocity
increased or decreased with the applied voltage, i.e.
with the electric field intensity. This result suggests that
the electric field change mainly causes the velocity
change. Moreover, comparison of the electric field
direction (X component) and the field-gradient direction
(Y component) confirmed that the dielectrophoretic force
is the main mechanism underlying the velocity change.
Similarly to Figure 10, the Y component in Figure 11
also shows movement in the opposite direction when the
electric field is cut off. This movement is apparently a
reaction in the viscous fluid when the force ceases acting
on the blood cells. As presented in Figure 11, movement
in the electric field direction (X component) also
increases and decreases as the applied voltage increases
and decreases. This movement is unlikely to be
electrophoresis, considering the viscosity of blood and
the period of commercial AC. Because this movement
corresponds well with the magnitude of the Y compo-

dent, we infer that it is attributable to the swirling motion
of the blood cells associated with movement in the Y
direction and its recoil return. In any case, it is inferred to
be measurement noise that differs from the motion of
interest.

Figure 12 presents a comparison between the
exposed electric field intensity and the change in
velocity, i.e. the dose–response relation. This result is
obtained from measuring the velocity of RBCs
observed in the central part of the observation area
in Figure 8 while varying the applied electric field.
The electric field on the symmetry axis in the
electrode arrangement in Figure 8 is proportional to
the voltage applied in the horizontal direction.

At a horizontal distance of 10 mm between
electrodes, we were able to observe the RBC move-
ment at voltages as small as 20 V, i.e. electric field
intensity of 2.0 kV/m. Moreover, in contrast to the
linear dependence shown in Figure 7, the
dose–response showed correlation with the square of
the applied voltage. As described in Chapter 2, the
driving force of dielectrophoresis is proportional to
the gradient of the squared electric field intensity.
Therefore, this result supports that the change in blood
cell velocity associated with AC electric field
exposure is attributable to dielectrophoresis.

CONCLUSIONS

Strong ELF electric fields are generated around
high-power electrical equipment such as high-voltage
power lines and electric railroads [ICNIRP, 2010;
Polk, 2017]. We investigated their effects on blood
cell movement as part of the clarification of their
biological effects. Thereby, we developed an electrode
system that exposes the electric field to blood, which
made it possible to measure RBC movement in
undiluted whole blood. The electrodes were designed
to have a constant electric field and a constant gradient
of electric field intensity in one direction, which
enabled us to separate electrophoresis and dielectro-
phoresis. Results demonstrate that, in whole blood,
electrophoresis was observed in the DC electric field
exposure from the field intensity of 0.40 kV/m and
dielectrophoresis was observed in ELF field exposure
from 2.0 kV/m.
These values are 10^3–10^5 times greater than the basic restrictions of 0.02–0.8 V/m for the public as stipulated in ICNIRP guidelines [ICNIRP, 2010]. Assuming Cassonian flow, the shear stress for RBC velocity 1.00 μm/s was estimated as 7.92 mPa for 0.07 Pa-s viscosity. This is almost 2–3 orders of magnitude less than the shear stress from aorta to capillaries, and 1–2 orders less than those in veins. Therefore, these study results raise no new issues in terms of human safety. Nevertheless, the level at which the effect occurs must be clarified because the electric field might be concentrated and enhanced in sharp areas of external shapes. Unlike general electrophoresis and dielectrophoresis measurements, this report is the first study that elucidates effects of specified electric field intensities under whole blood and commercial frequency conditions. Although the conditions used for this study do not match those in the blood vessel completely, the possibility of electric field effects on whole blood was verified in vitro. The next step is further investigation of this effect under conditions simulating an in vivo environment such as blood vessels, pulsatile flow under blood pressure, and body temperature.

In addition, future research must be undertaken to assess the physiological effects of blood cell movement changes associated with electric field exposure. Various possible applications of these findings include blood circulation promotion and local blood flow control. Furthermore, because blood cell movement depends on the electric field intensity in the body, such movement can be one justification for using the electric field intensity in the body as a basic parameter when setting safety standards. Further studies undertaken according to the perspectives presented above must be conducted in the future.

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