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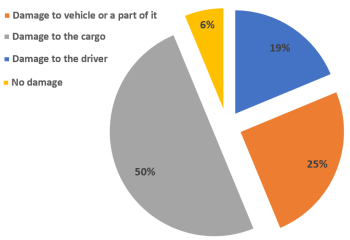

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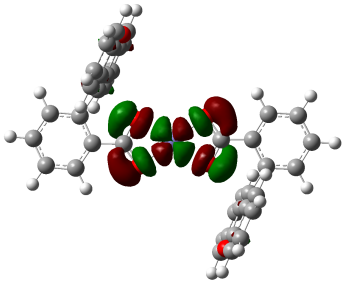

Core Problems the Road Freight Transport Companies Face due to the Refugee Crisis

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Abstract. The World Economic Crisis is still on the rise and has increased such processes as poverty, discrimination and war. In 2015 this phenomenon brought to Europe one of the biggest social-economic problem, called European Migrant crisis or European refugee crisis. According to Eurostat, EU member states received over 1.39 million first-time asylum applications in 2015 and this statistics does not even include the refugees who did not try to attain the asylum-seeker status.
Long waiting periods for the asylum permissions, asylum application cancellations, and humans' fears not to be accepted by the government's decisions, force people to take illegal actions entering the other countries, thus increasing illegal migration.
One of the most important features of this current migration process is application of various transportation means, modes and technologies which facilitates the human movement in time, space and speed. These illegal intrusions have been noticed all over the world in all transport types, such as air, land (rail, road) and water. Meanwhile, the European migrant crisis, evaluating the migrants who have already arrived in Europe, mainly enter illegal land transportations by road and rail freight transport, ignoring the rest.
In 2015 European countries recognized the illegal migration problem and improved the border control. During 2015, 1.82 million people were detained and arrested while attempting to cross the borders of the EU states illegally. The interruptions to road freight transport unit might disrupt the work of global supply chain and cause chain reaction malfunction to all supply chain participants.
The purpose of the research described in this paper is to highlight the relevance of the danger the illegal immigrants cause to the road freight transport units and the potential disruption to the global supply chains.

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Short title: Refugees impact on transportation.

Introduction

The World Economic Crisis is still on the rise and has increased such processes as poverty, discrimination and war. Therefore, many people from Africa, Middle East and Asia started immigrating to Europe. In 2015 this cause brought one of the biggest social-economic problems to Europe called European Migrant crisis, or European refugee crisis.

According Eurostat, EU member states received over 1.39 million first-time asylum applications in 2015 and these statistics do not even include the refugees who did not try to attain the asylum-seeker status.

Long waiting periods for the asylum permissions, asylum application cancellations, and peoples' fears to be unaccepted by the governments' decisions, force people to take illegal actions to enter other countries and increase illegal migration.

The purpose of the research described in this paper is to highlight the relevance of the danger that the illegal immigrants cause to the road freight transport units and the potential disruption to the global supply chains.

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1. The role of freight transportation in Supply Chain

Transportation is one of the most important factors in the supply chain and in the global economy, as it is a tool for moving the product from less useful place to more useful pace, creating added value, changing the product location as needed.

The transportation is a part of logistics and it is the main link between all of the elements of the Supply Chain - see Fig.1. Logistics requires planning and transportation is the key to execute the plan made, when getting freight from point A to point B. Meanwhile, as it is executive branch, it plays a big role in all the chain, as movement of the goods is needed

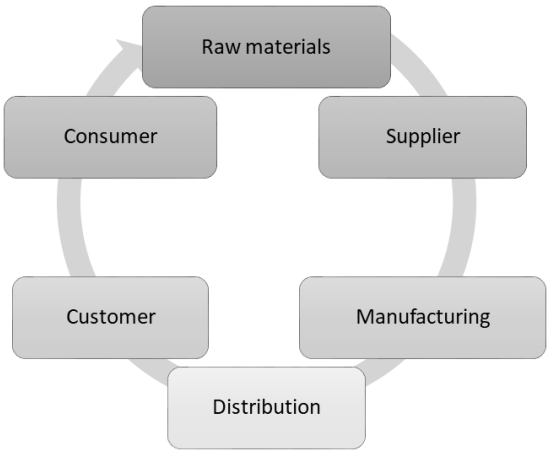

Fig. 1. Transportation link
between all the elements of Supply Chain.

to all members of supply chain, beginning from the raw materials and finishing with reverse logistics from consumers to recyclers (back to raw materials) – partially closed loop. Transportation link between all the elements of Supply Chain is presented in Fig. 1.

Transportation plays a connective role among the several steps that result in the conversion of resources into useful goods in the name of the ultimate consumer. It is the planning of all these functions and sub-functions into a system of goods movement to minimize the cost, maximize service to the customers that constitutes the concept of business logistics [1].

The role that transportation plays in logistics system is more complex than carrying goods for the proprietors. Its complexity can take effect only through high quality management. If transport system is well-handled, goods can be sent to the right place at the right time to satisfy customers' demands.

Therefore, transportation is the base of efficiency and economy in business logistics and expands other functions of logistics system. In addition, a good transport system performing in logistics activities brings benefits not only to service quality, but also to the company's competitiveness.

Land logistics is a very important link for logistics activities. It extends the delivery services for air and maritime transport from airports and seaports. The most positive characteristic of land logistics is the high accessibility level in land areas.

Land transport has advantages such as cheaper investment funds, high accessibility, mobility and availability. Its disadvantages are low capacity, lower safety, and slower speed. The excessive usage of land transport also brings many problems, for instance traffic jams, pollution traffic crashes and freight safety.

Today, international trade is commonplace (especially in EU) and increasing market share in emerging markets is highly desirable. For this purpose, more and more supply chain managers chose road freight transport as the main product transfer unit.

Road freight transport is the only transport type, which ensures door to door transportation. Compared to the other transportation methods (sea, rail, air), road transport used worldwide has less limits of path restrictions. It is much more flexible, and is the best solution for meeting price and speed ratio.

However, road freight transportation is one of the most unsafe methods for moving goods. It faces a lot of dynamic risks. Any disruption can significantly impact the financial and operational health of a transportation business and lead to the interference of the global supply chain and economy.

2. Risks in the supply chain, transportation and the human factor role in it

In the global supply chain, one of the most significant factors is time, and any disruption might cause a chain delay in all supply chain participants, especially when the disruption touches transportation - the supply chain connecting process. It is very important to avoid any triggers and evaluate all potential risks, to keep the supply chain effective and productive.

The longer the supply chain, the more important is the reliability and security factor. Since all the elements are closely interconnected any problem that arises in one part of the chain, might destroy the entire supply chain [2].

Supply chain management processes can be divided into the following levels: i) development of strategy; ii) planning; iii) execution of operations.

Transportation plays significant role in common supply chain at all levels mentioned above, and in case of unforeseen circumstances it can lead to interruptions to the entire supply chain. The most difficult task here is to eliminate various types of interruptions at the stage of operation, in order to avoid this, planning must include aspects of risk assessment and prevention that must be taken into account in each process at all levels of the supply chain [3].

There are basically two kinds of risks:

1) internal risks that appear in normal operations, such as late deliveries, excess stock, poor forecast, minor accidents, etc;
2) external risks that come from outside the supply chain, such as earthquakes, hurricanes, terrorist attacks and crimes.

Internal risks can be controlled by internal staff, and external risks are beyond managers' control. Managers cannot change the risk, but they can design operations that work as efficiently as possible within a risky environment [4].

The supply chain logistics must be sufficiently flexible to provide a restorative step when the failure approaches. The most important logistics missions in the supply chain are maintaining the quality by manoeuvring different logistical processes and quickly removing disturbances.

By focusing on a logistics mission in the supply chain,

flexibility must contribute to active readiness for the "optimal" decision, in other words to solve any disturbances in the supply chain [5]. Emergencies, events such as strikes, bankruptcies, earthquakes, etc. can break the supply chain or reduce its capacity. Different threats can affect the efficiency of the chain and lead to a rise of operations costs.

Risks and threats can be divided into accidental and intentional. Accidental is unpredictable action, which might be caused by various internal or environmental factors, which happen unexpectedly. Meanwhile intentional - is well planned action, usually thoughtfull by human with a certain purpose [5]. Security and risk cannot be perceived, and prevention cannot be planned if there is insufficient knowledge of an ongoing processes and threats.

Human aspirations always extend the limits of human perception. In order to understand the threats and risks that can lead to supply chain failures, it is important to draw up a vulnerability analysis in each level of supply chain [6].

Operational risk is associated with inherent uncertainty, such as unclear customer needs, unclear supply planning, costs or fluctuations in demand. The worst risk of disruption is the one which is unpredictable, as natural or man-made disruption, disaster or catastrophe [7].

According to Ref. [5], overall risks arise from the following sources as presented in Fig. 2:

1) environmental and industry factors;
2) organizational and problem-specific factors;
3) decision-maker factors.

The human factor is common to all categories. The human factor - determines human decisive solution, limited by his psychological and physical possibilities, and expressed through his behaviour or attitude. The human factor consists of:

i) human physiology;
ii) psychology (including perception, understanding, memory, social interaction, mistakes, etc.);
iii) environmental conditions;
iv) anthropometry (a branch of science about human body dimensions, e.g. strength) [8].

Since humans are capable of thinking and not just relying on their instincts, they are unpredictable. The unpredictability means the inability to control the future actions or ongoing processes. Environmental conditions are one of the most important aspects of human thinking. While discussing the disruptions in transportation operations, caused by external, intentional human activity, it is important to note that the main source is environmental risk factor.

According to Ref. [9], environmental risk factor might be subdivided into additional subcategories: a) political; b) policy; c) macroeconomic; d) social.

Social and political unrest is the result of a human factor, peoples' beliefs, and values, attitudes that do not reflect current government policies or business practices [10].

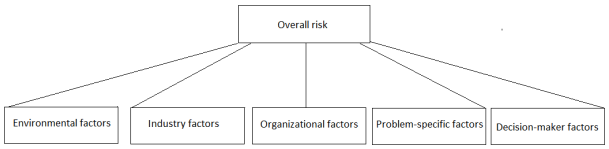

Fig. 2. Overall risk sources. Adapted according to Ref. [5].

Social unrest is a precursor and basis for further political turmoil. A new form of social unrest can be defined as a threat to the supply chain through terrorism and crime [11]. Terrorism and crime are the precisely planned intentional human actions, which are contradicts the law. These illegal actions are dangerous and might damage or stop the supply chain, bringing unpredictable damage.

People, who are most likely to commit a crime usually, are the ones who do not possess any assets and, therefore, often feel like they have nothing to lose. Such people are usually confused not being aware of what behaviour is socially acceptable and are psychologically unstable.

This instability is caused by the lack of basics from Abraham Maslow's (1970) hierarchy of needs [12]. If a person is hungry, does not have a shelter or feels threatened, he/she is ready for anything, without taking into consideration whether the actions are right or wrong, legal or not.

Illegal actions made by human factor might lead to the disruptions of supply chain, and must be evaluated as source of risk.

3. The relation between EU migrant crisis and the road freight transport

The European migrant crisis or the European refugee crisis is a term given to a period beginning in 2015 when rising numbers of people arrived in the European Union (EU). Asylum seekers are amongst these people, as well as a small part of economic migrants.

There are two types of migration: voluntary migration and forced migration.

Voluntary migration is based on an internal system of self-motivation, and it depends on how strongly individuals convince themselves that migration will improve the quality of their life. It identifies voluntary migration as a factor for social development and progress, which can bring benefits to both - the political and the administrative local unit, for example: contributing to the country's GDP growth, etc.

Meanwhile, forced migration is caused by an individual's psychological fears due to certain factors surrounding the environment that threaten the well-being or life. People, who have experienced psychological or physical abuse, feel forced to migrate and the psychics of such people is often impaired. Their desire to live differently influences the decision to migrate under any conditions; many of which go beyond certain laws and law enforcement boundaries, often associated with criminology.

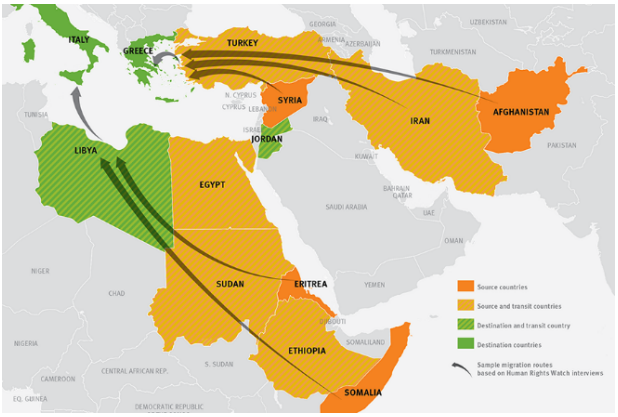

Fig. 3. Main migrants paths to the EU.
Adapted according to Ref. [13].

These individuals are difficult to predict and that can lead to various difficulties, which can cause much damage to both political and administrative units [14].

When the discussion comes to EU migrant crisis rather than referring to economic migrants who are looking for better lives, it discusses people, who try to save their lives (such as refugees and asylum seekers). The root reasons for forced migration, were hidden in 2007 world-wide financial crisis, civil wars in the South-East (Syria, Iraque, Iran, Afghanistan, Pakistan, etc.), and natural disasters (drought, floods, earthquakes, hurricanes, etc.) in Africa (Lake Chad Basin, South Sudan, Somalia, etc.). All these factors brought hunger, poverty, discrimination, persecution and wars. By the UN Refugee agency, 65.3 million people, or 1 person from set of 113 were displaced from their homes by conflict and persecution in 2015. This number of people movement is the highest level since World War II.

There are two types of peoples' migration: illegal migration (taking risk by themselves with various options); people smuggling (paying somebody to get to the target destination). And there are determined two main paths, which are used by migrants to get to Europe: the Mediterranean Sea (sea-transport); South-Eastern Europe road (land-transport).

Fig. 3 represents main migrants paths to the EU. Most of the migrants came from the Muslim-majority countries of regions in the South and East of Europe, as well as Western Asia, South Asia and Africa [15]. According to the United Nations High Commissioner for Refugees, the main countries from which refugees comes to the EU are Syria, Afghanistan, Somalia, Sudan, South-Sudan, Congo Democratic Republic, Central African Republic, Iraq, Eritrea, European countries which are mainly reached by refugees first are Italy and Greece. It is due to a comfortable geographic position, between South-East Europe and Africa.

It is important to note that Italy and Greece are just the first stop as the purpose is to move to the countries with better social benefits and familiar languages.

According to Eurostat (2016) EU member states received over 1.39 million first-time asylum applications in 2015, more than double that of the previous year. Five states Germany, Hungary, Sweden, Austria, and Great Britain) received around two-thirds of the EU's asylum applications in 2015 [16].

Assessing the number of registered asylum applications and the number of registered refugees arriving to Europe from 2008 to 2015, it can be noted that not all arrivals submit applications for asylum. This leads to the conclusion that those, who did not submit asylum applications, will settle in and stay in the country illegally or hide from the law migrating between European countries, using illegal methods. Fig. 4 represents illegal refugees' movements inside EU.

In a large part of the EU - the Schengen area - people can move freely without being checked at internal borders, but refugee flows have forced some Member States to reintroduce passport checks at borders with other EU countries. Illegal migration inside Europe reached a critical level.

After improved border control in 2015, 1.82 million people were detained and arrested while attempting to cross the borders of the EU states illegally. 95% of illegal entries to EU were by sea transport, and 5% by land transport (road and rail) [18].

Even if migrants use different first paths of migration to the EU (Mediterranean Sea or South Eastern), once they reach Europe, everyone travels using land transport, as it is essential and connective movement unit between EU countries.

Heavy land transport such as trucks and trains, gives a possibility for refugees to hide with a hope not to be caught at the borders and easily move from one country to the other.

As countries border, officers inspect only suspicious vehicles which meet specific risk criteria (each country has its own assessment of risk criteria) capable of transporting refugees, most of them successfully cross border sections and continue moving in Europe towards their targeted country. Checking all vehicles would be much more effective, there would be an excessive waiting line at the borders, and the number of civil servants on the border would increase resulting in more additional costs for the States. However, if this were to be done, there would be more precision and more precise assessment of such phenomenon [18].

Fig. 4. Illegal refugees' movements inside EU.
Adapted according to Ref. [17].

Illegal migrant movements inside the EU have not been defined, as not all migrants are seized at borders and not all of them have the same ultimate destination of the country which means that the movement down the roads and corridors are as well different.

4. Research

This research was devoted for indentifying the core problems caused by refugees to road freight transport companies in FR-UK corridor. Anonymous questionnaire was submitted to 53 international road freight transport companies which work with cargo transportation on FR-GB corridor. 36 respondents replied to all survey questions.

The FR-GB corridor as a research object was chosen because of a high problematic situation with refugees. France Cale harbor refugee camp is one of the biggest refugee camps in Europe. Harbor location is comfortable as there are a lot of trucks waiting for transportation by ferries to the UK. According to "Help Refugees" organization statistics by 2016 in Cale refugee camp had been living 5497 refugees. Even after refugee camp demolition, in October 2016, the refugees came back to the previous place and renewed the settlement. The number of residents in the camp has been increasing.

Huge number of refugees is like a windbreak. When they come in front of trucks in the street, the vehicles are slowing down the speed, trying not to run over the people, but the slower speed gives a chance to enter into a moving lorry and hide in the trailer among the products.

The safety around Cale port is weak as well. It is not recommended for drivers to stop for a rest or refuel their trucks within 100 km radius from Cale.

The purpose of this research is to show what kind of demurrage and losses are caused by the refugees' getting on trucks. What kind of consequences it might bring to international transportation companies – as to business units, to countries' economies and even to the global supply chain.

Main reasons why refugees are getting on trucks:

1) "door to door delivery";
2) more stops, which are unprotected, not observed;
3) easier to fight with one or two drivers than with a squad of officers on the border;
4) easier to hide, closing the trailer doors or sewing the tilt;
5) higher chance not to be noticed among production inside the truck;
6) food trailers guarantee the "satisfying trip".

The results of the research revealed that 92% of European international freight companies which had participated in the research, had incidents involving refugee intrusions. Even the companies, which transportation volume on exploratory direction (FR-UK) was up to 20% of the total cargo volume.

Illegal migrants are taking various actions to get to the desired destination: violent, aggressive intrusions; secret

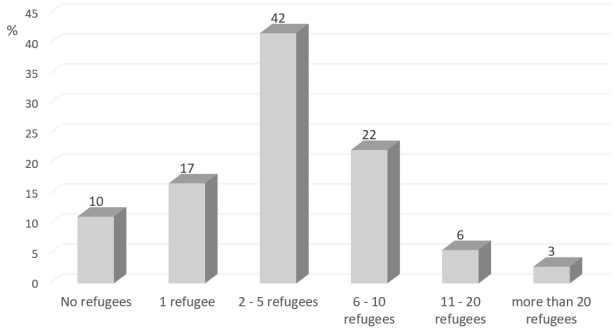

Fig. 5. The number of refugees per one truck intrusion.

intrusions; trying to bribe a driver.

Participating transport companies in the questionnaire confirmed that 72% of the intrusions were done secretly.

Most incidents with refugees were an organized group intrusions rather than solitary acts. Group intrusions took over around 73%. Among the most popular are the intrusions by the group of 2÷5 persons (42%) and the group of 6÷10 refugees (22%) - see Fig. 5.

The respondents have noted, that in 2014, they had only 5% of incidents with refugees, comparing to the period of 2014÷2016. Most of these intrusions hit European freight transport companies in 2015 (53%) during the European Refugee Crisis. However, in 2016, the frequency of intrusions remained rather intense, up to 42%.

In 2016, 12 respondent companies encountered 20-40 intrusions per year, 6 companies 40 to 60 intrusions per year, 3 companies between 60÷100 intruders in 2016 and 15 companies had fewer than 20 incidents with refugees per year.

Just for comparison in 2014 there were 16 companies which had no incidents with refugees, meanwhile in 2015, there were only 2 companies, and in 2016 – 6 freight transport companies who had no accidents with refugees. Fig. 6 represents the distribution of frequency of refugee intrusions to the road freight transport units in 2014÷2016.

Even 89% of Europe's international freight companies have suffered losses. The biggest losses incurred by companies were €50,000 per incident with refugees, while the smallest one less than €500 (not counting companies that managed to flail collisions with refugees), in any case, while dealing with refugees, it is impossible to avoid financial and

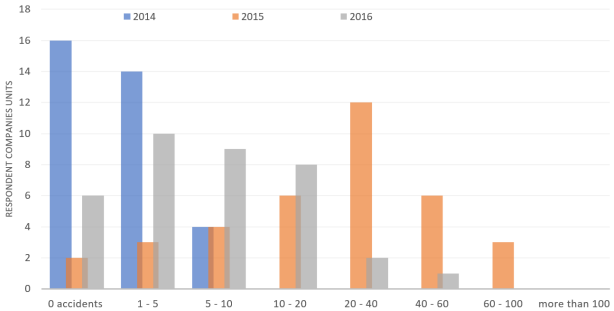

Fig. 6. Frequency of refugee intrusions to the road freight transport units in 2014÷2016.

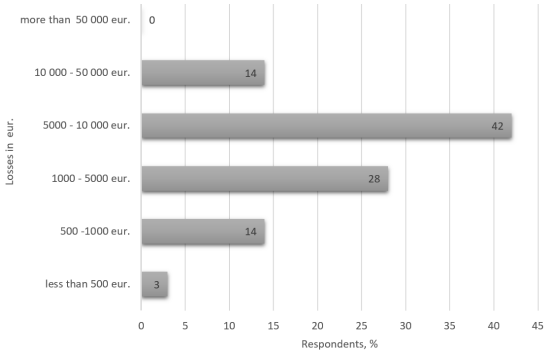

Fig. 7. Losses incurred by road freight transport companies because of collision with refugees.

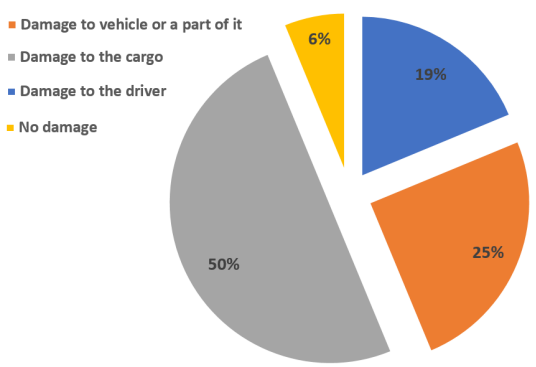

Fig. 8. Types of damages.

material losses - see Fig. 7.

Types of damages could be classified into three groups as presented in Fig. 8:

1) cargo damage, at around 50%;
2) damage to vehicle or a part of it - 25%;
3) damage of a driver - 19%.

The consequences after collision with illegal immigrants were categorized by all companies similarly: most of them suffered from financial losses and faced cargo transportation insurance increase on the FR-GB corridor. Meanwhile, some companies faced the termination of collaboration, because they could not protect the goods from the threat. Fig. 9 represents the distribution of consequences after collision with illegal migrants.

Refugees were usually found during the border check by officers or during the transportation by drivers. Most invasions occur in unprotected areas (45%) and during the slow movement across the border (25%). It is difficult to determine the time of getting on the truck because respondents' answers were quite similar - 39% at night, 30% at day time and 31% did not know the answer. From a location point of view, the riskiest European transit countries are: France, Germany and the Netherlands.

Preventive measures have been applied by 81% of companies, but only 17% provided more detailed information on preventive measures, such as company internal documents and technical measures.

Even with preventive measures applied, the percentage of interruptions to the road freight transport units still reaches 92%. It means that the measures which are used are not effective.

It should be taken into the consideration how to solve this problem. It is necessary to evaluate the situation and new to apply risk assessment models.

Conclusions

International road freight transport has many different transportation corridors. As it is shown in this paper, first arrivals to the EU come to Italy and Greece. The secondary movements of refugees inside Europe might be foreseen by the asylum applications statistics but it is only a conjecture, as not all of the arrivals apply for an asylum, some of them arrive when decisions to move illegally had already been made.

Illegal migrants damage the trailers and the trucks during the break in. Refugees are using trucks as transportation means but while staying inside the trailer full of products they damage the goods, contaminate it, or destroy it whilst being there. In cases where food is being transported and any kind of human-being evidence is found, the whole cargo is supposed to be utilized and all the losses must be covered by international cargo Transportation Company.

The hold of the load or utilization of the goods cause chain delays in the whole supply chain and can affect the economy. If it happens more frequently, it will affect the whole global supply chain and the restoration might lead to higher expenses and time-loss on a global scale. This problem must be solved.

According to Eurostat (2015), the statistical agency estimates that in 2036, the number of refugee arrivals to the EU will reach 1.5 million / year. Looking ahead, the European refugee crisis will only become a bigger problem, as the conflict zones will move to civilian war zones and it will force people to migrate. It is forecasted that from 2016 to 2036, the number of refugees in Europe will increase exponentially. Since 2036÷2068, the number of refugees will not be smaller than in 2015.

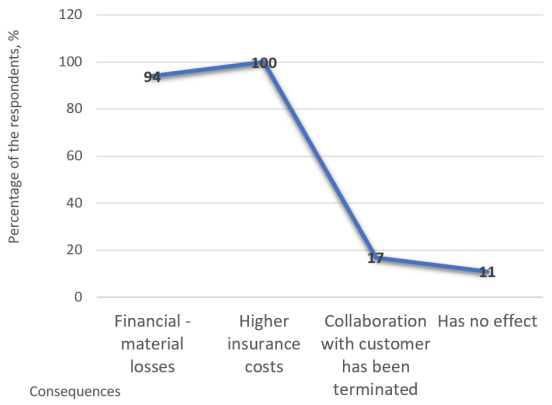

Fig. 9. Consequences after collision with illegal migrants.

This paper shows the nowadays situation, and if this Eurostat prognosis will be more or less right, Europe will face a huge challenge managing the Europe's refugee crisis of the century.

This research is a small step towards opening the discussion and encouraging the academic society to act and show that this problem must be solved not only on a social, but also on a political level and human migration risk assessment models, which would prevent the negative effect on business units, countries, economies and interruptions of the supply change caused by refugee migration must be created.

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Mechanism of Effective Quenching of Calcein Fluorescence by Iron. *Ab initio* study

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Abstract. Geometry of the associate of the calcein and iron ion was determined using density functional CAM-B3LYP method. A stable associate was obtained only as set of a two calcein molecules and iron ion. The nature of the calcein luminescence extinguished by Fe^{2+} ions is explained by the formation of low-lying eight additional electronic states, when the transitions from the above states to the ground states are completely forbidden due to the heavy metal Fe^{2+} effect. Energetical scheme (in the form of Jablonski diagram) is illustrated by plots of charge redistribution between one-particle states which are related to spectrometric states.

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Keywords: Fluorescence quenching; Calcein; Ferum ion; Ion-pair complex.
Short title: Quenching of calcein fluorescence.

Introduction

Cell electroporation represents the process when the permeability of the cell plasma membrane temporary increases due to strong electrical field pulse (up to 300 kV/cm). As an invasive method, cell electroporation is widely used in cell biology, biotechnology, and medicine [1,2]. The application of high voltage in the electrolyte medium, despite other electrolytic reactions, involves the oxidation of the metal of anode. For this reason, the anode begins to melt [3].

Metal ions emitted from electrodes can react with fluorescent molecules and reduce their fluorescence intensity. One of the most popular materials used in the production of electrodes and applied to cell electroporation is stainless steel. Usage of high-voltage pulses creates a substantial number of iron ions (Fe^{2+} and Fe^{3+}) from the anode [4].

Calcein - a well-known derivative of fluorescein - belongs to the class of fluorescent dyes. It is widely used for the determination of the permeabilization of biological membranes [5,6] including cell electroporation studies [7,8].

Influence of iron ions on the intensity of calcein fluorescence was observed in various media, e.g. HEPES-buffered saline, cytoplasm-like solution or serum [9,10]. The results of this work may be useful in evaluating the effectiveness of cell electroporation [11].

The aim of this work was to study *in silico* the structure and energetical properties of calcein derivatives in surroundings where significant amounts of Fe^{2+} are present:

a) to determine the most probable geometry of associates consisting of calcein molecule and iron ion Fe^{2+};
b) to explain the mechanism of quenching of calcein luminescence.

1. Literature review

Fluorescence is a molecular process of spontaneous emission of radiation, which is not related to the thermal equilibrium with its environment [12-15]. Emission could occur from electronically excited state. Excitation must be provided by external source, for example, laser, LED etc. in case if excitation wavelength corresponds to the wavelength interval of absorption band.

Fluorophores (fluorescent probes) are extremally stable molecular derivatives that absorb light of a certain wavelength and emit it at longer wavelengths - so called Stokes shift occurs [13,15]. Main reason of such phenomenon is related to the processes of internal conversion when non-radiative transitions occur [14].

The usage of fluorescent probes allows solving the visualization problems: fluorescent probes have a high contrast to dark background, high sensitivity and specificity. Fluorophores are usually minimally-invasive compounds that can be present in live cells for real-time exposition without cell fixation [16].

Fluorescence imaging belongs to one of the most important non-invasive methods in contemporary biological scien-

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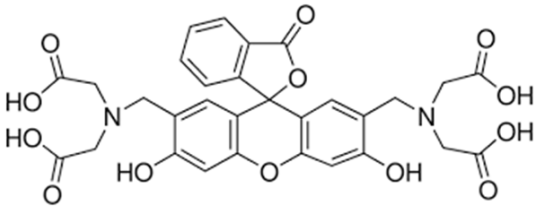


Fig. 1. Calcein green.

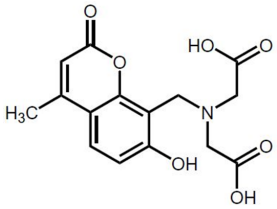


Fig. 2. Calcein blue.

ces [13]. High sensitivity of the method allows precise usage for medical needs, working with unknown biological structures [16]. From a physical point of view, several factors are important for occurrence of the fluorescence images:

i) quantum energy and intensity of excitation source;
ii) stability and quantum efficiency of fluorophore [17];
iii) presence of quenchers and agents of resonant energy transfer [18];
iv) presence of (photo)oxidation reagents in the medium.

2. Chemical structures

Calcein green [19] and calcein blue [20] are dyes for visualization purposes *in vitro* with high luminescence yield - see Figs. 1-2. Due to high molecular stability, calcein plays a role of short-term label in the cell with excitation and emission at 495/515 nm (2.5/2.4 eV), respectively [21].

Acetoxymethyl (AM) esters of calcein green and calcein blue are non-fluorescent compounds, which can freely pass though the cellular membrane into live cells. The active esterases within live cells cleave AM group and calcein-AM is transformed into a bright fluorescent form – calcein, which is membrane-impermeant [22]. Such an approach is used to recognize viable cells because dead cells either do not have the intact plasma membrane or do not contain active enzyme esterases that can hydrolyze calcein–AM.

3. Quantum chemistry simulations

Quantum-chemical structure simulations were performed with *Gaussian16* package [23]. Molecular geometry was optimized by density functional CAM-B3LYP method using a 6-31G(d) base with polarizing d functions without environmental impact. Fig. 3 represents the model derivative M1 corresponding to a complex of two calcein molecules and an Fe^{2+} ion. In order to simplify the ground state energy minimization procedure, calcein structure has been used without long-chain substitutions, but it is still called as "calcein". Three projections allow to imagine the nature of bonding through the iron ion. Due to the sp^2 hybridization, the bonding is formed in a plane containing a calcein phenyl ring with carbonyl. The intermolecular bridge is formed as a double behaviour of co-ordinate bonds [..C=O−Fe^{2+}−O=C..] and [..C=O→Fe^{2+}←O=C..].

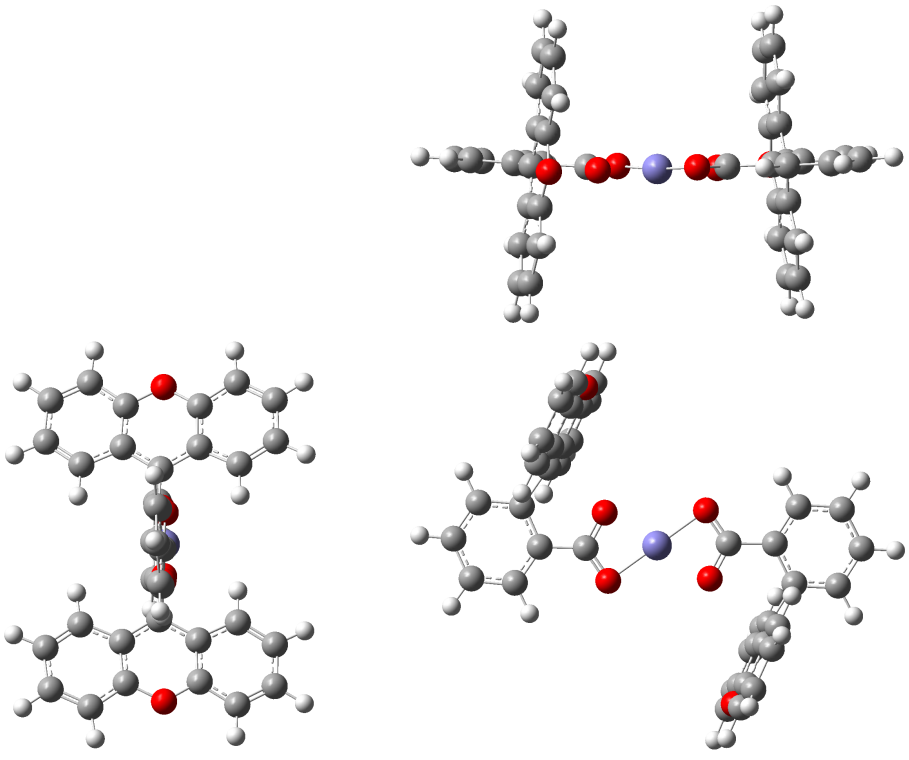


Fig. 3. A model derivative M1 as the complex of two calcein molecules and an Fe^{2+} ion (at centrum). X0Z, X0Y, Y0Z projections.

Table 1. Electronic transitions of simulated absorption spectrum of model compound M1.
Gaussian16[23], semiempirical method TD, only singlets included.

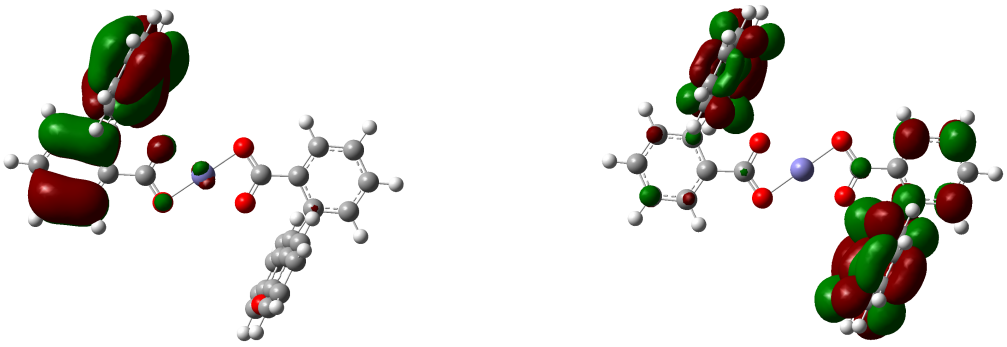
Electronic transition	Energy, eV	Oscillator strength	One-particle transitions	Coef.	Figs.
$E_0 \rightarrow E_1$	0.495	0.000	167B→170B	-0.43	
			167B→177B	-0.42	
			167B→179B	-0.70	4
$E_0 \rightarrow E_2$	0.571	0.000	**157A→174A**	-0.53	5
			161A→174A	-0.51	5
			164B→182B	-0.53	5
			167B→182B	-0.40	
$E_0 \rightarrow E_3$	0.579	0.000	164B→181B	-0.63	
			167B→181B	-0.76	6
$E_0 \rightarrow E_4$	0.949	0.000	157A→174A	0.42	
			161A→174A	-0.49	7
			164B→182B	0.45	
			167B→182B	-0.44	
$E_0 \rightarrow E_5$	1.075	0.000	164B→170B	-0.40	
			164B→179B	-0.64	8
$E_0 \rightarrow E_6$	1.569	0.000	**164B→181B**	0.70	9
			167B→181B	-0.59	
$E_0 \rightarrow E_7$	1.893	0.000	**157A→174A**	-0.66	10
			164B→182B	0.57	
$E_0 \rightarrow E_8$	2.220	0.000	168A→170A	-0.62	
			165B→168B	0.64	11

The electronic excitations were calculated by means of semi-empirical TD method for singlets only (number of states is equal to 10). Table 1 shows the simulated electron absorption spectrum of the model compound M1. Parameters of spectroscopic states (excitation energy and oscillator strength) as well as corresponding one-particle transitions are presented in order to estimate the population of excited states from lowest excited E_1 up to E_8. Set of transitions could be divided into three groups: transitions in the band group about 0.5 eV, band group about 1 eV, band group in region 1.5÷2 eV. Other transitions into higher states are not significant. The limit of about 2 eV was chosen because the fluorescence of the iron-free calcein derivatives is observed from the state about 2.31 eV, and the iron ions quench that luminescence very effectively. In this case, eight excited electronic states below 2.3 eV are related to the quenching processes.

Typically, the eight lowest transitions are completely forbidden (oscillator strength of transition is equal to zero). Hence, a radiative transition (fluorescence emission) is impossible, only internal relaxation processes take place. The molecular state at 2.31 eV is not populated, and the state E_1 at 0.49 eV must be titled as the lowest excited singlet state. Since the transition $E_0 \rightarrow E_1$ is forbidden (and also $E_1 \rightarrow E_0$), there is no luminescence.

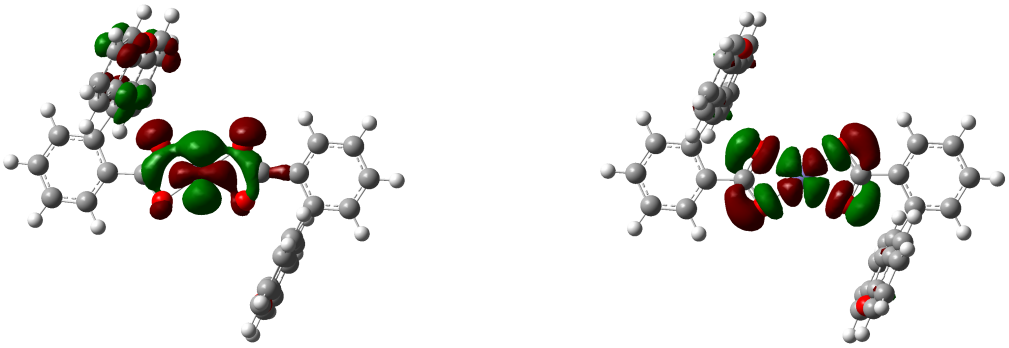
Tables 4÷11 show the redistributions of electronic clouds by population of excited states E_n, n=1÷8. All charge redistributions are depicted in the framework of MO related to the one-particle transition. Most important one-particle transition could be divided into three groups.

For low lying states E_n, n=1,2,3, (see Figs. 4÷6), two types of intermolecular redistribution occur. Firstly, transition between MO 167B→179B (from left to right calcein, see

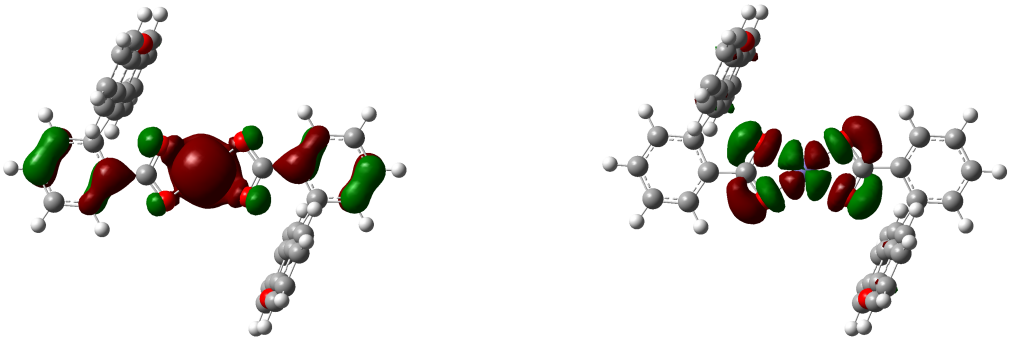


Charge redistribution between MO: **167B→179B**

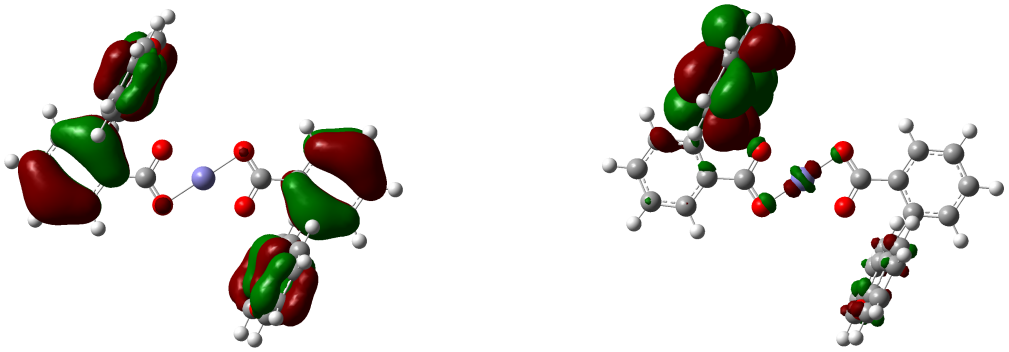
Fig. 4. Electronic transition $E_0 \rightarrow E_1$, transition energy 0.495 eV, oscillator strength 0.000.



Charge redistribution between MO: **157A→174A**

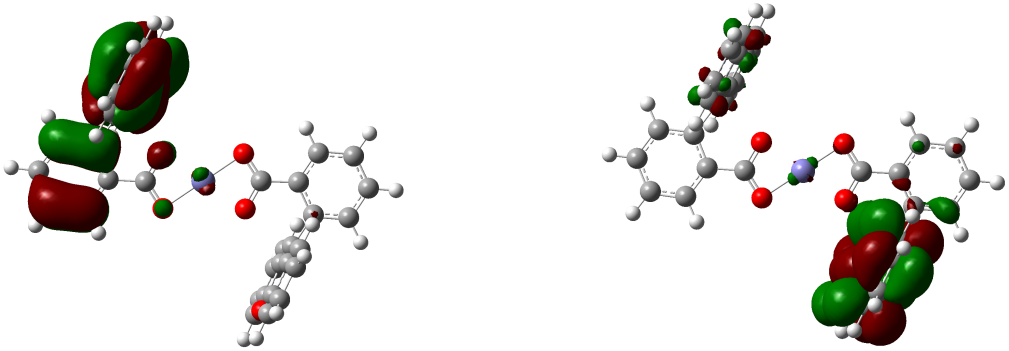


Charge redistribution between MO: **161A→174A**



Charge redistribution between MO: **164B→182B**

Fig. 5. Electronic transition $E_0 \rightarrow E_2$, transition energy 0.571 eV, oscillator strength 0.000.

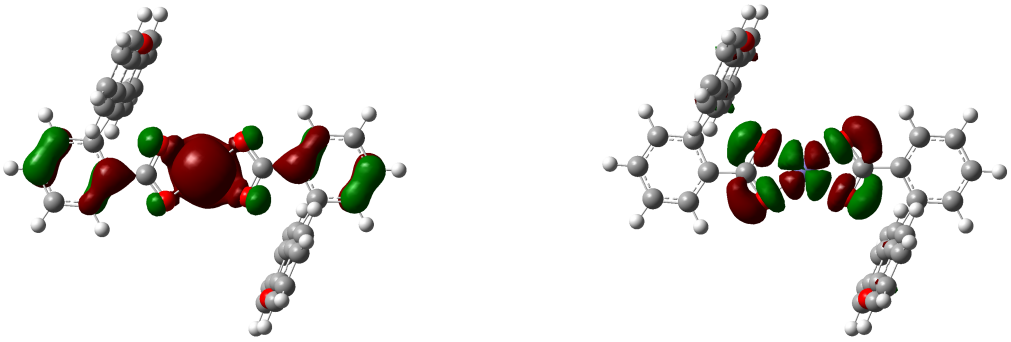


Charge redistribution between MO: **167B→181B**

Fig. 6. Electronic transition $E_0 \rightarrow E_3$, transition energy 0.579 eV, oscillator strength 0.000.

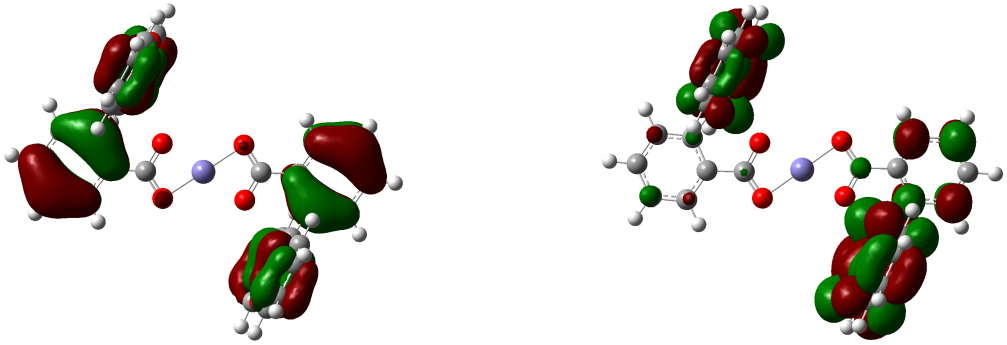
Fig. 4) as $\pi \rightarrow \pi^*$ transition is typical for such type redistributions. Iron ion plays the role of the mediator in order to establish the bridge behaviour for inter-fragmental redistribution. The same type is realized for transition between MO 167B→181B (from left calcein to right calcein, see Fig. 6). Secondly, transitions between MO 157A→174A and 161A→174A (from iron ion and oxygen-carbon atoms in near surrounding to iron ion with neighbour oxygens, see Fig. 5) must be treated as mixed transition with $n \rightarrow \pi^*$ impact (n electrons from oxygen atoms).

For low intermediate states E_n, n=4,5,6, (see Figs. 7÷9), two types of intermolecular redistribution occur. Firstly, tran-



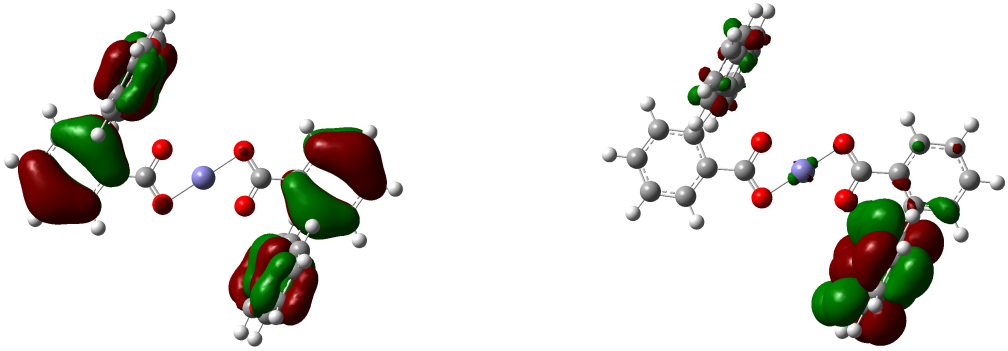
Charge redistribution between MO: **161A→174A**

Fig. 7. Electronic transition $E_0 \rightarrow E_4$, transition energy 0.949 eV, oscillator strength 0.000.



Charge redistribution between MO: **164B→179B**

Fig. 8. Electronic transition $E_0 \rightarrow E_5$, transition energy 1.075 eV, oscillator strength 0.000.



Charge redistribution between MO: **164B→181B**

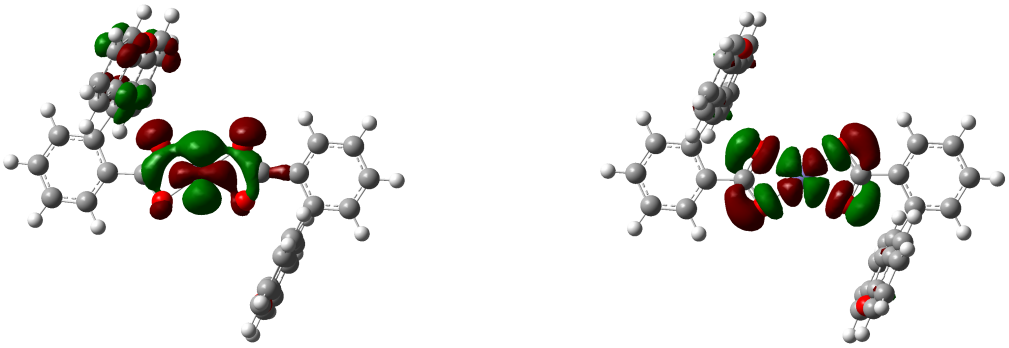
Fig. 9. Electronic transition $E_0 \rightarrow E_6$, transition energy 1.569 eV, oscillator strength 0.000.

sition between MO 164B→179B (from left to right calcein, see Fig. 8) as $\pi \rightarrow \pi^*$ transition is typical for such type redistributions. The same type is realized for transition between MO 164B→181B (from left calcein to right calcein, see Fig. 9). It is necessary to point out that full CT transition is not realized in both cases. Secondly, transitions between MO 161A→174A (from iron ion and oxygen-carbon atoms in plane surface to iron ion with neighbour oxygens in the same plane surface, see Fig. 7) must be treated as mixed transition with $n \rightarrow \pi^*$ impact.

For high energy states E_n, n=7,8, (see Figs. 10÷11), two types of intermolecular redistribution occur. Firstly, transitions between MO 157A→174A (from iron ion and oxygen-carbon atoms in plane surface to iron ion with neighbour oxygens in the same plane surface, see Fig. 10) must be treated as mixed transition with $n \rightarrow \pi^*$ impact.

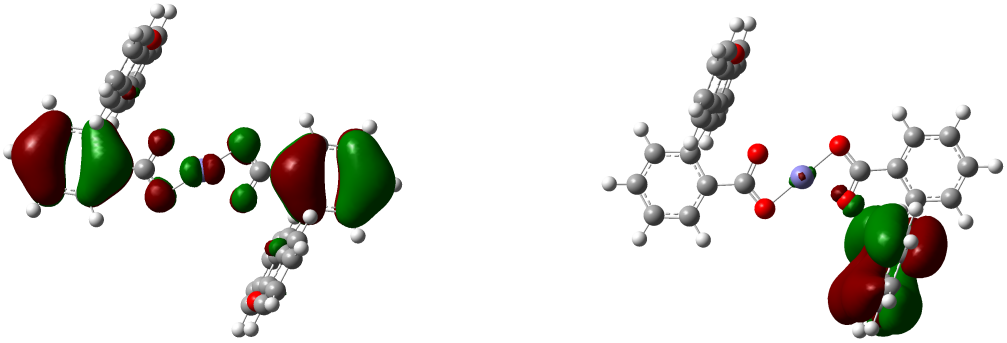
Secondly, new type of charge redistribution occurs for transition between MO 165B→168B (from plane surface containing two calcein molecules to right calcein, see Fig. 11). This transition of mixed type is forbidden due to orthogonality condition (fragments are perpendicular).

Fig. 12 represents Jablonski diagram explaining the quenching effect of calcein fluorescence due to iron ions. For iron-free calcein (left diagram), absorption and luminescence are present as radiative transitions $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_0$, respectively. Quenching of luminescence is absent. For M1 compound containing Fe^{2+} (right diagram), transitions $S_n \rightarrow S_0$, n=1÷8, are forbidden (oscillator strengths are equal to zero), luminescence (emission) is impossible. According



Charge redistribution between MO: **157A→174A**

Fig. 10. Electronic transition $E_0 \rightarrow E_7$, transition energy 1.893 eV, oscillator strength 0.000.



Charge redistribution between MO: **165B→168B**

Fig. 11. Electronic transition $E_0 \rightarrow E_8$, transition energy 2.220 eV, oscillator strength 0.000.

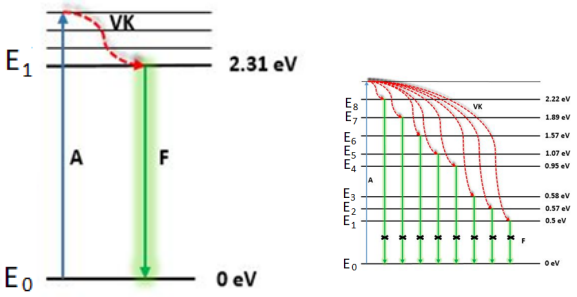


Fig. 12. Jablonski diagram for iron-free calcein (left, allowed emission) and M1 compound (right, forbidden emission).

to Franck–Condon principle, depopulation of the excited states could be realized through non-radiative processes of internal conversion.

It is necessary to point out that the state E_1 (most important for quenching process) is present in infra-red region (2.5μm/0.5 eV).

4. Conclusions

The geometry of calcein and iron ion associate was investigated by simulations using CAM-B3LYP/6-31G(d) method.

The stable structure was obtained only for associate consisting of two calcein molecules and the iron ion.

The nature of calcein luminescence extinguishing with Fe^{2+} ions was explained by the formation of eight additional electronic states, when transitions from excited states to the ground state are completely forbidden due to the heavy metal (Fe^{2+}) effect.

Three types of charge redistribution between MO were established:

a) intermolecular between two calcein molecules;
b) "intramolecular" in near Fe^{2+} surrounding including neighbour oxygen atoms;
c) intermolecular between two perpendicular plane surfaces from calcein to calcein.

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