RAPORT STIINTIFIC

privind implementarea proiectului ***PN-III-P4-ID-PCE-2016-0007,***
contract nr. 6/2017
Etapa 1

Conform programului experimental prevăzut în acest proiect, obiectivele principale şi activităţile de cercetare aferente acestei etape pot fi redate unitar astfel: **Pertracţia sinergică a acizilor rozmarinic şi pantotenic**.

Activităţile prevăzute au fost realizate în totalitate şi au inclus studiile dedicate:

1) Pertracţia sinergică a acizilor rozmarinic şi pantotenic din soluţii pure;

2) Pertracţia sinergică a acizilor rozmarinic şi pantotenic din lichide de fermentaţie simulate;

3) Pertracţia sinergică a acizilor rozmarinic şi pantotenic din lichide de fermentaţie reale.

In continuare va fi prezentată o sinteză a rezultatelor experimentale derulate în acestă etapă a proiectului.

Având în vedere faptul că rezultatele experimentale incluse în etapa 1 a proiectului au constituit subiectele unor lucrări publicate sau acceptate spre publicare în reviste cotate ISI, raportul va fi redactat în limba engleză, respectând informaţiile originale din articolele menţionate.

The efficiency of a reactive extraction system can be improved by using a mixture of extractants or an extractant and a phase modifier, a process known as synergic reactive extraction. Mainly, the synergic effect is the result of two simultaneous phenomena:

- changing the individual extraction capacity of extractants
- changing the structure of molecular species extracted.

Thus, one extractant reacts with the solute forming a solute - extractant complex, while the other one increases this complex hydrophobicity by solvation, while the structural configuration of extractants does not generate sterically hinders. The use of extractants and solvent mixtures in the organic phase generally leads to increased extraction efficiency and higher yields compared to those obtained from the individual use of extractans or solvents.

Rosmarinic acid possesses a complex structure having multiple reaction centers that can react with the extractants considered in the extraction systems. Previous studies have indicated that irrespective of the nature of the extractant, the reactive extraction of rosmarinic acid is based on the formation, at the interface between the aqueous and organic phases, of a highly hydrophobic compound. The mechanism of the interfacial reaction, its kinetics, and the conditions for carrying out the reactive extraction depends on the type of extractant used and the polarity of the organic phase.

Since rosmarinic acid contains highly basic phenolic groups in the structure, it can react with acidic extractants such as D2EHPA. In this case, reactive extraction takes place through an ion exchange interfacial reaction, the solute being in protonated form occurring at low pH-value of the aqueous phase:

$$RA\ (O^{+}H_2)_{2(aq)} + 2HP_{(o)} \rightleftharpoons RA\ (O^{+}H_2)_2P_2^{-}{}_{(o)} + 2H^{+}_{(aq)}$$

($RA\ (O^{+}H_2)_{2(aq)}$ symbolizes the rosmarinic acid with two active phenolic groups and HP the organophosphorus extractant).

The strong acidic domain determines also the protonation of the extractant which becomes unable to react with rosmarinic acid. Due to these opposite phenomena appearing at an acidic pH domain, the maximum yield for reactive extraction of the rosmarinic acid with D2EHPA is reached at pH = 3. This mechanism of reactive extraction of rosmarinic acid with D2EHPA is also supported by the variation of the distribution coefficient with the extractant concentration, for the two solvents used, shown in Figure 1.

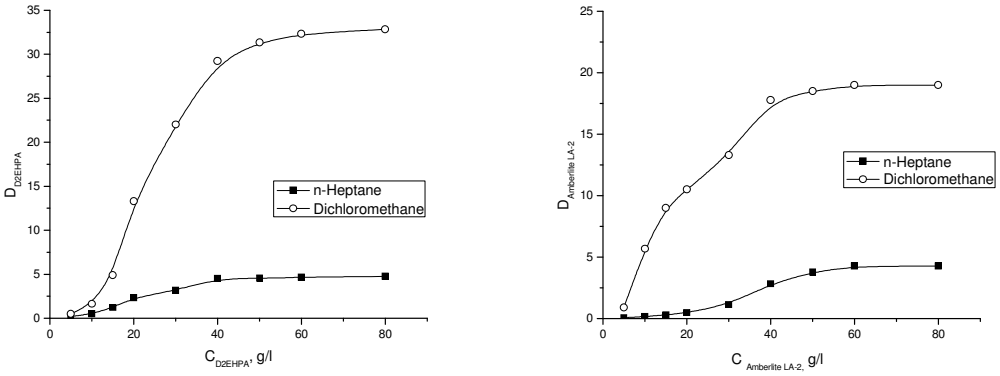


Figure 1. Influence of D2EHPA concentration on the distribution coefficient of rosmarinic acid.

The presence of the carboxyl group in the structure of the rosmarinic acid allows the reactive extraction with Amberlite LA-2 (Q), the process occuring by means of the interfacial interactions between the two components. The interfacial interactions could be of the hydrogen-bonding type if the rosmarinic acid is undissociated in the aqueous phase or of the ionic type in the case of acid dissociation:

$$RA\text{-}COOH\ (aq) + n\ Q\ (o) \rightleftharpoons RA\text{-}COOH.Qn\ (o)$$

Depending on the structural characteristics of the extraction system components, as well as, on the polarity of the solvent, acidic or aminic adducts could be formed at the interface. The voluminous molecule of rosmarinic acid induces steric hindrances, an effect associated with lower initial concentration of the acid compared to that of the aminic extractant. This leads to the formation of interfacial adducts of either ammonium salt type, formed by neutralizing the carboxylic group of the solute with one molecule of extractant, or of aminic type if n ≥ 2. In the above context, the reactive extraction of rosmarinic acid with Amberlite LA-2 follows the next mechanisms of the interfacial reaction:

- *extraction system with n-heptane*

$$RA\text{-}COOH_{(aq)} + 4\ Q_{(o)} \rightleftharpoons RA\text{-}COOH.Q_{4\,(o)}$$

- *extraction system with dichloromethane*

$$RA\text{-}COOH_{(aq)\ )} + Q_{(o)} \rightleftharpoons RA\text{-}COOH.Q_{(o)}$$

These mechanisms indicate that the structure of the interfacial compound depends on the polarity of the solvent. Thus, reactive extraction with Amberlite LA-2 dissolved in the solvent with low polarity (n-heptane) results in the interfacial formation of an aminic adduct involving four molecules of extractant. In contrast, if the solvent with higher polarity (dichloromethane) is used, one molecule of each component of the extraction system is involved in the formation of the interfacial compound.

The influence of the aminic extractant concentration on the rosmarinic acid distribution coefficient for the used solvents is shown in Figure 2.

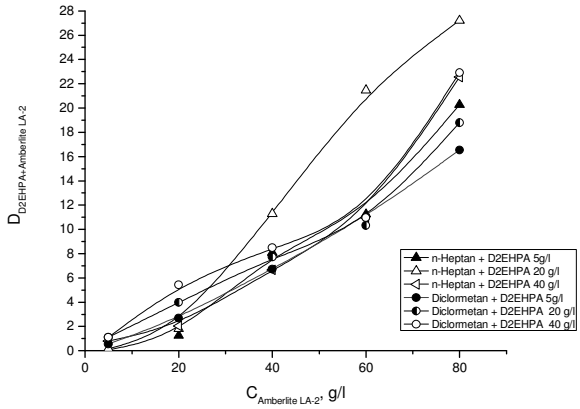


Figure 2. Influence of Amberlite LA-2 concentration on the distribution coefficient of rosmarinic acid.

Compared to reactive extraction with D2EHPA, it can be observed that the differences between the two solvents are maintained, the distribution coefficient reaching high values for the solvent with higher polarity, exhibiting a strong increase for Amberlite LA-2 concentrations up to 40 g/l.

The above results, cumulated with our previous studies on synergic reactive extraction of carboxylic acids indicated the possibility to obtain a synergic effect by using a mixture of Amberlite LA-2 and D2EHPA for reactive extraction of rosmarinic acid.

For verifying the potential synergic effect of the extractants mixture, the variations of synergic coefficient with the concentration of Amberlite LA-2 and D2EHPA, respectively, in the mixture have been plotted in Figure 3.

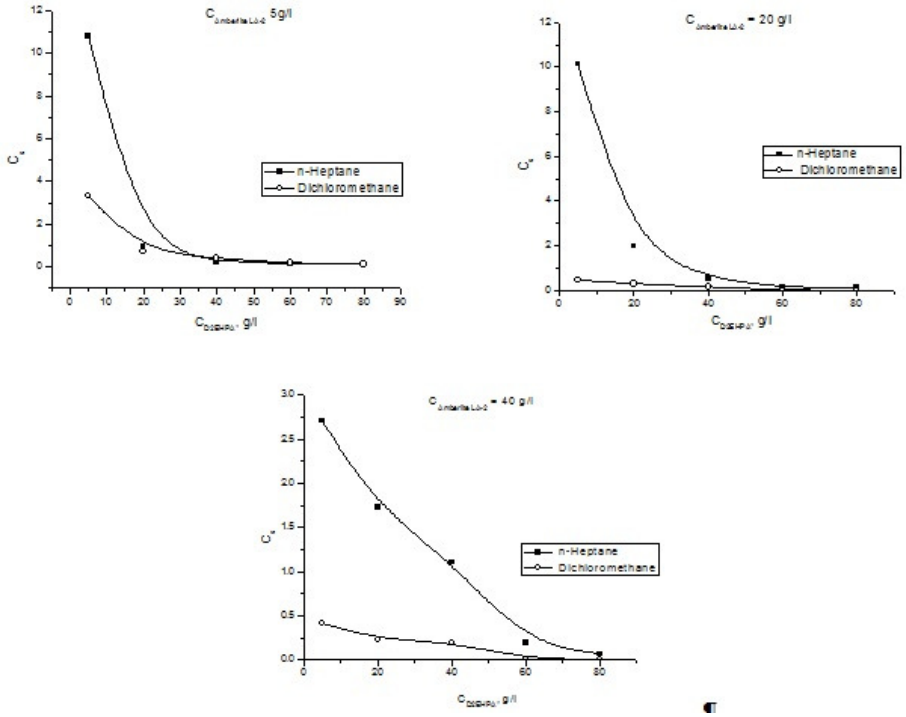


Figure 3. Influence of Amberlite LA-2 concentration on rosmarinic acid distribution coefficient for the synergic reactive extraction system (pH = 2).

The synergic effect of the extractant mixture can be estimated using the synergic coefficient defined with the following expression:

$$C_S = \frac{D_{D2EHPA+LA-2}}{D_{D2EHPA} + D_{LA-2}}$$

the synergis being achieved for $C_s > 1$.

By graphical representation of the experimental data for reactive extraction systems with extractants mixture, maintaining either Amberlite LA-2 or D2EHPA concentration at constant values, the influence of each extractant on the synergic effect can be determined.

Figures 5 indicate that the values of the synergistic coefficient are higher for the solvent with lower polarity, namely n-heptane. For a constant concentration of Amberlite LA-2 in the organic phase, the increase of D2EHPA concentration leads to decrease of the synergistic coefficient. As mentioned above, the increase of D2EHPA concentration prevents the formation of amine adducts and, consequently, affects the hydrophobicity of the interfacial complex with rosmarinic acid. For these reasons, the diminution of C_S is more important for dichloromethane. This effect is additionally confirmed by the recorded values of C_S for 20 g/l and 40 g/l Amberlite LA-2, which are lower than 1 for the entire considered domain of D2EHPA concentration (negative synergism).

In the reactive extraction system with a constant concentration of D2EHPA in the organic phase, C_S increases with the increase of Amberlite LA-2 concentration (Figure 6). Depending on the solvent polarity and D2EHPA amount in the solvent phase, the synergic effect is observed for Amberlite LA-2 concentrations over 20 g/l dissolved in n-heptane. Thus, by increasing the concentration of D2EHPA from 5 to 40 g/l, the minimum concentration value of Amberlite LA-2 corresponding to synergism increases from 20 to 40 g/l for the solvent with lower polarity, namely, n-heptane, while there is no synergic effect recorded for dichloromethane. The results suggest that Amberlite LA-2 contributes to the formation of aminic adducts by solvating the complex produced by interfacial reaction between rosmarinic acid and D2EHPA.

Based on the results obtained for studies on individual extraction with Amberlite LA-2 and D2EHPA respectively, and those obtained for the extraction with these extractants mixture, the following interfacial mechanism was considered, at the formation of interfacial complexes at participating two molecules of organophosphoric (HP) extractant and n molecules of aminic extractant (Q):

$$HOOC\text{-}RA(O^+H_2)_{2(aq)} + 2\,HP_{(o)} + n\,Q \rightleftharpoons Qn.HOOC\text{-}RA(O^+H_2)_2\,P^-_{2\,(o)} + 2\,H^+_{(aq)}$$

The distribution coefficient for synergic reactive extraction of rosmarinic acid can be determined with the relationship:

$$D_{D2+LA-2} = \frac{\left[Qn.HOOC - RA\left(O^+H_2\right)_2 P^-_{2\,(o)}\right]}{\left[HOOC - RA\left(O^+H_2\right)_{2(aq)}\right]}$$

where $[HOOC\text{-}RA(O^+H_2)_{2(aq)}]$ and $[Qn.HOOC\text{-}RA(O^+H_2)_2P^-_{2(o)}]$ represent the overall concentrations of rosmarinic acid and the extracted compound at the equilibrium state in the related phase.

According to the interfacial equilibrium, the extraction constant, K_E, can be calculated with the following expression:

$$K_E = \frac{\left[Qn.HOOC - RA\left(O^+H_2\right)_2 P^-_{2\,(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[HOOC - RA\left(O^+H_2\right)_{2(aq)}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]^n}$$

in which the concentrations of the constituents are expressed in moles/l.

The synergic coefficient can be expressed according to concentrations of the components of extraction system at the equilibrium:

$$C_S = \frac{D_{D2EHPA+LA-2}}{D_{D2EHPA} + D_{LA-2}} = \frac{\dfrac{\left[Qn.HOOC - RA\left(O^+H_2\right)_2 P^-_{2\,(o)}\right]}{\left[HOOC - RA\left(O^+H_2\right)_{2(aq)}\right]}}{\dfrac{\left[HOOC - RA\left(O^+H_2\right)_2 P^-_{2\,(o)}\right]}{\left[HOOC - RA\left(O^+H_2\right)_{2(aq)}\right]} + \dfrac{\left[Qn.HOOC - RA\left(O^+H_2\right)_{2\,(o)}\right]}{\left[HOOC - RA\left(O^+H_2\right)_{2(aq)}\right]}} =$$

$$= \frac{\left[Qn.HOOC - RA\left(O^+H_2\right)_2 P^-_{2\,(o)}\right]}{\left[HOOC - RA\left(O^+H_2\right)_2 P^-_{2\,(o)}\right] + \left[Qn.HOOC - RA\left(O^+H_2\right)_{2\,(o)}\right]}$$

By liniarizing the above equation, the following relation which represents the equation of a straight line is obtained:

$$ln\,\mathrm{K_E} + \mathrm{n}\,ln\left[\mathrm{Q}_{(o)}\right] = ln\,\mathrm{D}_{\mathrm{D2+LA-2}} + ln\left[\mathrm{H}^{+}_{(aq)}\right] + ln\left(\left[\mathrm{H}^{+}_{(aq)}\right] + \mathrm{K_{a1}}\right) - 2\,ln\left[\mathrm{HP}_{(o)}\right]$$

where $[Q_{(o)}]$ and $[HP_{(o)}]$ are the concentrations of the reactants free in the organic phase.

Graphical representation of this equation is presented in Figure 4 for constant values of the organophosphoric extractant and different concentrations of the aminic extractant for the extraction systems with n-heptane and dichloromethane. From the slope of the straight line it is possible to determine the number of Amberlite LA-2 molecules, n, which participate in the formation of the interfacial adduct, and from its intercept the value of the synergic extraction constant, K_E.

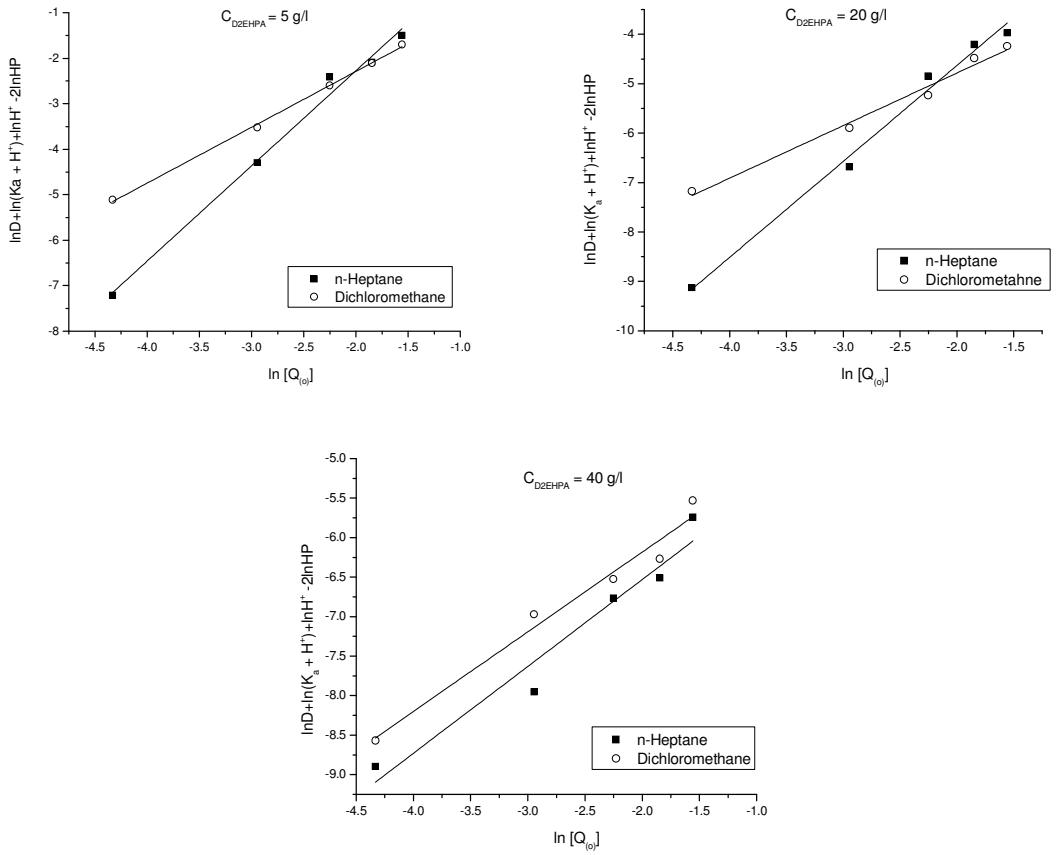


Figure 4. The graphical representation of the straight lines.

According to Figure 4, depending on D2EHPA concentration and the polarity of the solvent, the number of aminic extractant molecules involved in the interfacial adducts varies as follows:

- D2EHPA concentration 5 g/l
  - n-heptane n = 2.09
  - dichloromethane n = 1.12
- D2EHPA concentration 20 g/l
  - n-heptane n = 1.94
  - dichloromethane n = 1.06
- D2EHPA concentration 40 g/l
  - n-heptane n = 1.1
  - dichloromethane n = 1.0

These results indicate a dependance of the structure of the interfacial compound on the concentration of the organophosphoric extractant and the solvent polarity. Thus, in the case of n-heptane, two molecules of Amberlite LA-2 participate to the formation of aminic adducts for D2EHPA concentrations of 5 and 20 g/l ($Q_{2(o)}$.HOOC-RA$(O^+H_2)_2P^-_{2(o)}$), respectively one molecule for 40 g/l D2EHPA ($Q_{(o)}$.HOOC-RA$(O^+H_2)_2P^-_{2(o)}$), participate in the interfacial complex formation. For the extraction system with dichloromethane, the solvent with highest polarity, one molecule of aminic extractant is involved in the formation of the interfacial complex, irrespective of the concentration of the organophosphoric extractant.

The obtained data confirm the previous assumptions regarding the negative effect of the increased concentration of D2EHPA and of the solvent polarity on the number of Amberlite LA-2 molecules that contribute to the solvation of the product formed by the interfacial reaction between rosmarinic acid and D2EHPA and implicitly on the efficiency of synergic reactive extraction.

The values of the synergic extraction constant indicated in Table 1 are also dependent on the D2EHPA concentration and the polarity of the organic phase. Similarly to the synergic effect, this parameter is affected by the increase of D2EHPA concentration and the polarity of the solvent. This variation emphasis the direct dependence between the hydrophobicity of the interfacial complex, increased with the increase in the number of Amberlite LA-2 molecules involved in its formation, and the synergic extraction efficiency.

Table 3. Mathematical expressions and values of synergic extraction constants corresponding to studied systems.

C_{D2EHPA}, g/l	Solvent	Synergic extraction constant	Value
5	n-Heptane	$K_E=\frac{\left[Q_{2(o)}.HOOC-RA\left(O^+H_2\right)_2P^-_{2(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[\overline{HOOC-RA\left(O^+H_2\right)_{2(aq)}}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]^2}$	6.78 (l^2/mol^2)
	Dichloromethane	$K_E=\frac{\left[Q_{(o)}.HOOC-RA\left(O^+H_2\right)_2P^-_{2(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[\overline{HOOC-RA\left(O^+H_2\right)_{2(aq)}}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]^2}$	1.18 (l^2/mol^2)
20	n-Heptane	$K_E=\frac{\left[Q_{2(o)}.HOOC-RA\left(O^+H_2\right)_2P^-_{2(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[\overline{HOOC-RA\left(O^+H_2\right)_{2(aq)}}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]^2}$	$4.7\cdot10^{-1}$ (l^2/mol^2)
	Dichloromethane	$K_E=\frac{\left[Q_{(o)}.HOOC-RA\left(O^+H_2\right)_2P^-_{2(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[\overline{HOOC-RA\left(O^+H_2\right)_{2(aq)}}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]}$	$7.6\cdot10^{-2}$ (l/mol)
40	n-Heptane	$K_E=\frac{\left[Q_{(o)}.HOOC-RA\left(O^+H_2\right)_2P^-_{2(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[\overline{HOOC-RA\left(O^+H_2\right)_{2(aq)}}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]}$	$1.52\cdot10^{-2}$ (l/mol)
	Dichloromethane	$K_E=\frac{\left[Q_{(o)}.HOOC-RA\left(O^+H_2\right)_2P^-_{2(o)}\right]\cdot\left[H^+_{(aq)}\right]^2}{\left[\overline{HOOC-RA\left(O^+H_2\right)_{2(aq)}}\right]\cdot\left[HP_{(o)}\right]^2\cdot\left[Q_{(o)}\right]}$	$1.56\cdot10^{-2}$ (l/mol)

For the considered experimental conditions, by comparing the information from Table 1 with those previously discussed, it can be affirmed that the most efficient system for synergic reactive extraction of rosmarinic acid is that containing the extractants mixture dissolved in n-heptane, the concentration of D2EHPA in solvent being 5 g/l.

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Din punct de vedere al obiectivelor generale ale programului PCE-IDEI, **rezultatele obţinute** în cadrul proiectului până în au fost diseminate pe parcursul anului 2017 după cum urmează:

1) publicarea **1 articol** într-o revistă ISI şi acceptarea **1 articol** într-o revistă BDI (*incluzând acknowledgements*);

2) **5 participări** la conferinţe internaţionale;

3) includerea rezultatelor într-o **teză de doctorat** al unui membru al echipei de cercetare;

4) obţinerea unei **Medalii de aur**, a două **Medalii de argint** şi a unei **Medalii de Bronz** la Saloanele intenaţionale de Inventică;

5) obţinerea **Premiului pentru cel mai tânăr cercetător** de către un membru al echipei la Salonul Infoinvent Chişinău;

6) continuarea şi dezvoltarea colaborării cu alte universităţi din ţară (U.M.F. „Gr.T. Popa" din Iaşi).

Director proiect,
Prof.univ.dr.ing. Dan Caşcaval