MODIFIED POLYSACCHARIDES WITH AZOIC CHROMOPHORES
1. HYDROPHYLIC DIAMINODIPHENIL METHANE DYE

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An original strategy was set-up in order to develop new azo compounds which incorporate in their structure 4,4'-diaminodiphenylmethane (DADFM) and low molecular weight maltodextrine. The synthesis of the model compounds involved the functionalisation of DADFM with the maltodextrine, the conversion of amino functionalized maltodextrine in a diazonium salt and the coupling of reaction with H and R acids. The compounds were characterized by UV-VIS, IR, mass spectroscopy and thermal analysis. This new design unreported in literature will confer to the synthesized compounds a greater solubility, on the entire pH range, which will result in an extended application field of these dyes, on various substrate types.

INTRODUCTION

Most of the chemical compounds containing one or more diazo groups are designed as dyes or pigments and when present on a substrate they selectively modify the reflection or the transmission of incident light.[1] In the majority of dyeing processes the dye is transferred from aqueous solution onto the substrate, so a desirable property of a dye is an adequate aqueous solubility. For instance, many anionic dyes depend on their sulphonic or carboxylic acid groups for their solubility in water.[2] The cheapest way of solubilising a dyestuff is to introduce in its molecule one or more sulfonic groups. This process does not generally cause any diminish in the light fastness of the chromogen to which it is attached.[1,2] Strongly chromophores as natural anthocyanidins[3] or phthalocyanines[4] can be naturally attached to carbohydrates, or synthetically modified by attachement to carbohydrates in order to rise their solubility in water (eg. in the case of biological and medical applications such as photodynamic therapy or as catalyst in aqueous media). Due to the reorganization process at nanometric scale under UV/VIS irradiation, azo-containing polymers have attracted much attention as materials with applications in biology, optoelectronics and nano-manipulation field.[5] The introduction of a hydrophilic component of polysaccharide type in the design of an azo dye may result in an increase of solubility, the dye being soluble on the entire pH range. In this way, the technologic applicability of these dyes will be considerably greater, and may be extended to non-conventional fields of

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application. In this work, this concept as well as the strategy which was developed in order to reach this goal, are presented for the first time.

RESULTS AND DISCUSSION

Dyes derived from 4,4'-diaminodiphenyl-methane and/or from 4,4'-diaminobenzanilide derivatives may present interest in non-conventional area due to their flow birefringent properties, which were found as influencing parameters regarding the viscosity and temperature of dye solutions.[6-10]

An original strategy was set-up in order to develop azo dyes which incorporate in their structure 4,4'-diaminodiphenylmethane and some natural hydrophilic components. This involves the following three steps: the functionalisation of an aromatic diamine with a maltodextrine (Scheme 1); the separation of the unreacted maltodextrine from the functionalised one and from the excess of diamine, and finally the conversion of the aminated maltodextrin in a diazonium salt and the coupling of this intermediate compound with 1- and 2- naphthol derivatives, *e.g.* the 8-amino-1-naphthol-3,6-disulphonic acid (H acid) and 2-naphthol-3,6-disulphonic acid (R acid) (Scheme 2).

As model compounds for low molecular weight maltodextrine, Paselli MD 14, with a dextrose equivalent (DE) = 14 (Mn =1100) was chosen. As aromatic diamine, 4,4'-diamino diphenil methane (DADFM) was used. To create a new C–N bond between a polysaccharide and DADFM the reductive amination reaction was chosen. Although the reaction occurs better in a pH ranging from 6 to 8, the reaction has also been shown to successfully work at a pH as low as 4 and as high as 10.[11] A series of aromatic amines (as UV labels) was linked to oligosaccharides by reductive amination with an yield ranging from good to excellent: 2-aminopyridine, p-aminobenzoic acid, 4-aminobenzonitrile, and hexametilenediamine.[12,13] To achieve high conversion of polysaccharides, large excesses of DADFM were used in order to avoid the formation of diblock polysaccharides as a result of double reductive amination on the same DADFM molecule. The obtained DADFM-maltodextrine compound (DADFM-PAS14) was separated from uncoupled polysaccharides by ion exchange chromatography and purified by dialysis against water. The inspection by thin-layer chromatography (TLC) showed no presence of unreacted DADFM. An aqueous solution of DADFM-PAS14(0.04M) was subjected to UV-VIS

spectroscopy. The maximum of absorption was found at 240 nm, and it was noticed that the analyzed compound shows a slight bathochromic shift as compared to the maximum of absorption specific to the ethanolic solution of DADFM, which was found at 244.2 nm. This maximum of absorption indicates the presence of the aromatic moiety in DADFM-PAS14 (**1**, Scheme 1). The IR spectrum of DADFM-PAS14 which was performed in KBr, shows the specific vibrational motions of an aromatic ring (by instance 1577,0 cm^{-1}, 1411,6 cm^{-1}, 1151,3 cm^{-1}, 853.3 cm^{-1}, 759,8 cm^{-1}).[14] These results are in agreement with the assumption of the bonding between the aromatic component and the polysaccharidic one. Finally, DADFM-PAS14 was subjected to mass spectrometry analysis and the (+) ESI HCIT-MS spectrum is presented in Fig. 1.

The mass spectrum shows a rather high heterogeneity of the DADFM-PAS14 as illustrated by the large number of peaks molecular species, detected as singly charged ions with m/z ranging from 100–1600. The proeminent peaks are represented by a singly charged envelope of seven protonated molecules (m/z=525.2; 687.5; 849.3; 1011.4; 1173.5; 1335.6; 1497.7) separated by a mass interval of 162.0, the mass of the glucose repeat unit. These ions were assigned, based on the exact mass calculation, to DADFM-PAS14 species containing from one to seven glucose building blocks, in addition to the modifying reducing end moiety (Glc_1-Ag → Glc_7-Ag, Scheme 1).

Thus, the compound was considered as being pure enough to be further used in the next step (the diazotization reaction). As coupling components, 8-amino-1-naphthol-3,6-disulphonic acid (acid H) (**3**) and 2-naphthol-3,6-disulphonic acid (acid R) (**4**) (Scheme 2) were chosen, due to the fact that they are widely used, and have been proved their great importance since the early stage of the synthetic dyes history. Thus, R acid is one of the most energic coupling components of the naphthol sulfonic acids category, H acid presents a great versatility, which is conferred by the presence of both an amino and a hydroxy group. Both coupling components were used as their sodium salts. DADFM-PAS14 (**1**) was subjected to an usual diazotisation process in order to obtain its diazonium salt (**2**).[15] The coupling reaction of the diazonium salt (**2**) with (**3**) and (**4**) respectively, was carried out in alkaline medium, using a large excess of coupling component, in order to ensure that the diazonium salt was entirely used. The reaction products were dyes DADFM-PAS14HA (**5**) and, DADFM-PAS14RA (**6**) respectively. In the case of both coupling reactions, the excess of

coupling components (**3**) and (**4**) was removed from the reaction mixture, by size exclusion dialysis (membrane with MW/CO=1000). This purification method was successful and this matter of fact was strengthen by the results of the TLC analysis (AcOEt:MeOH=1:1 (v/v)), which was performed for each dye's dialyzate. Thus, the TLC analysis indicated in the case of dye (**5**) the absence of compound (**3**), and in the case of dye (**6**) the absence of compound (**4**). It is well known from literature that the coupling reaction of H acid (**3**) with the diazonium salt (**2**) occurs in the 2 position of H acid when the coupling reaction is performed in alkaline conditions. So there is strong evidence that the sulphonated aminonaphthols, exhibit a great versatility, due to the presence in the naphthalene ring of both an amino and a hydroxy group. This makes them among the most important group of azo intermediates, and among these, H acid can couple under the relevant conditions of pH in the 2 (alkaline conditions) or the 7 (acidic conditions) positions.[16] In the case of R acid, the coupling position in alkaline conditions is exclusively in the 4 position, as well known from literature.[17,18]
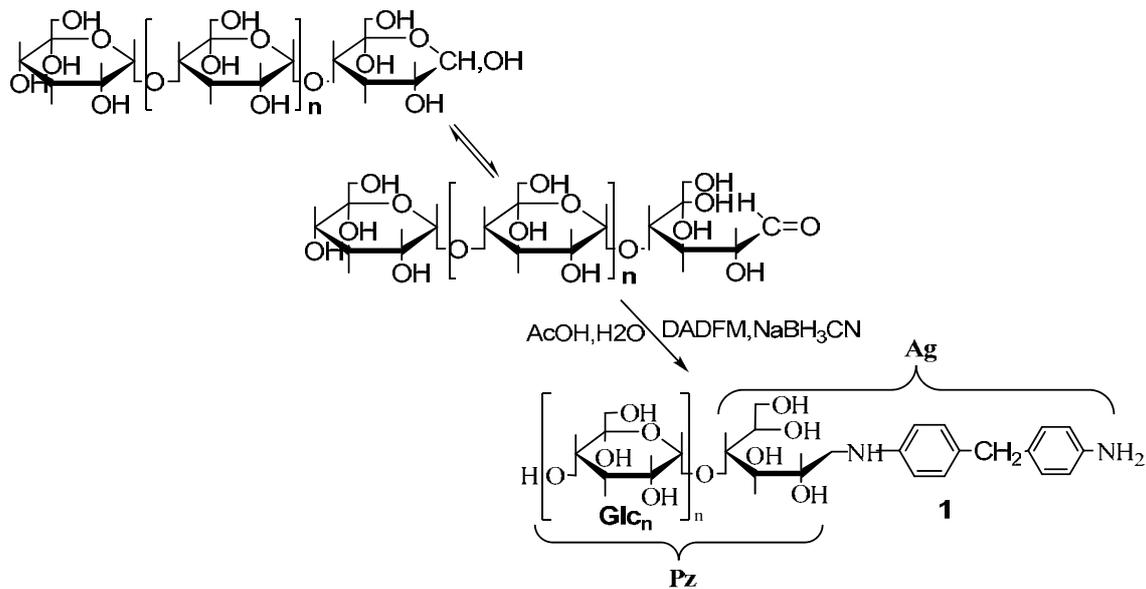
Scheme 1 – Synthesis of DADFM-linked polysaccharides: reductive amination of Paselli 14 with DADFM and sodium cyanoborohydride as reducing agent.
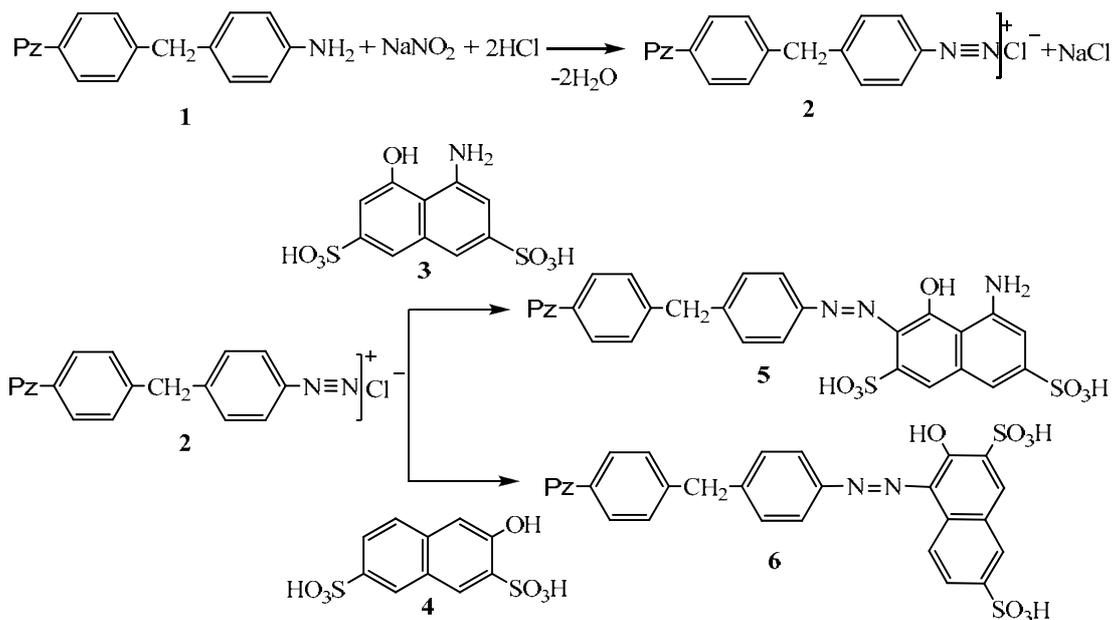
Scheme 2 – Synthesis of hydrophylic diaminodiphenil methane dyes.
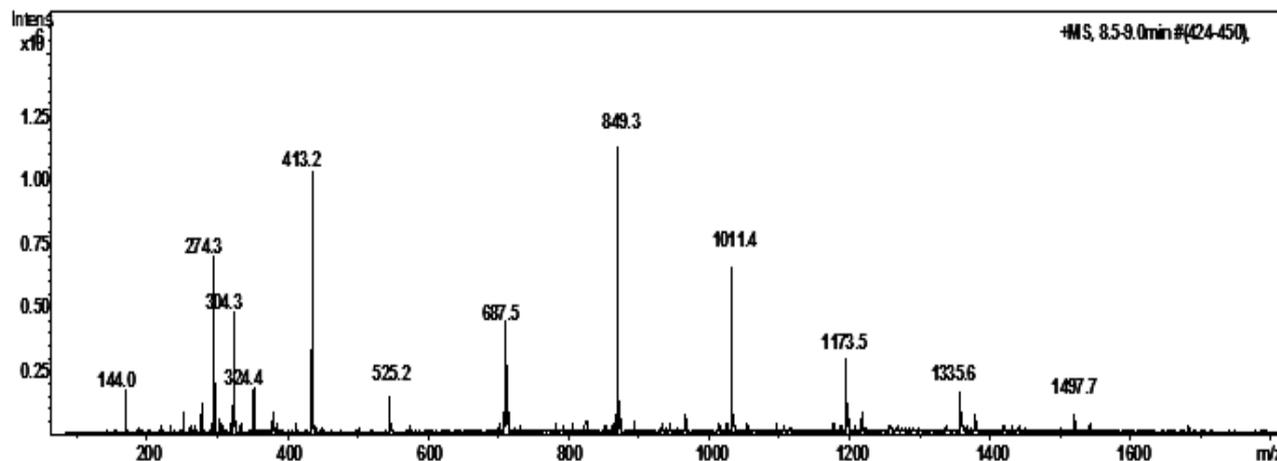
Fig. 1 – Mass spectrometry analysis; (+) nano-ESI-HCIT-MS of DADFM-PAS14.

Dyes (**5**) and (**6**) were analysed by IR spectroscopy (KBr pellets), and their aqueous solutions by UV-VIS spectroscopy (see Fig. 2).

The IR spectroscopic analysis of compounds (**5**) and (**6**) indicates the presence of the specific band of azo compounds, some other being also present in the reacting compounds. The specific bands of the azo's group (-N=N-), were found at 1506.0 cm^{-1} (**5**) and 1506 cm^{-1} (**6**) and are of medium or low intensity, as mentioned in the literature.[14, 19-21] But, only from IR data, one could not state without doubt, that compunds (**5**) and (**6**) were formed through synthesis. In Fig. 2, the UV-VIS spectra of compound (**5**) versus compounds (**1**) and (**3**) are shown. One could notice a well-marked maximum of absorption (547.0 nm), which is located in the visible domain only in the case of compound (**5**).
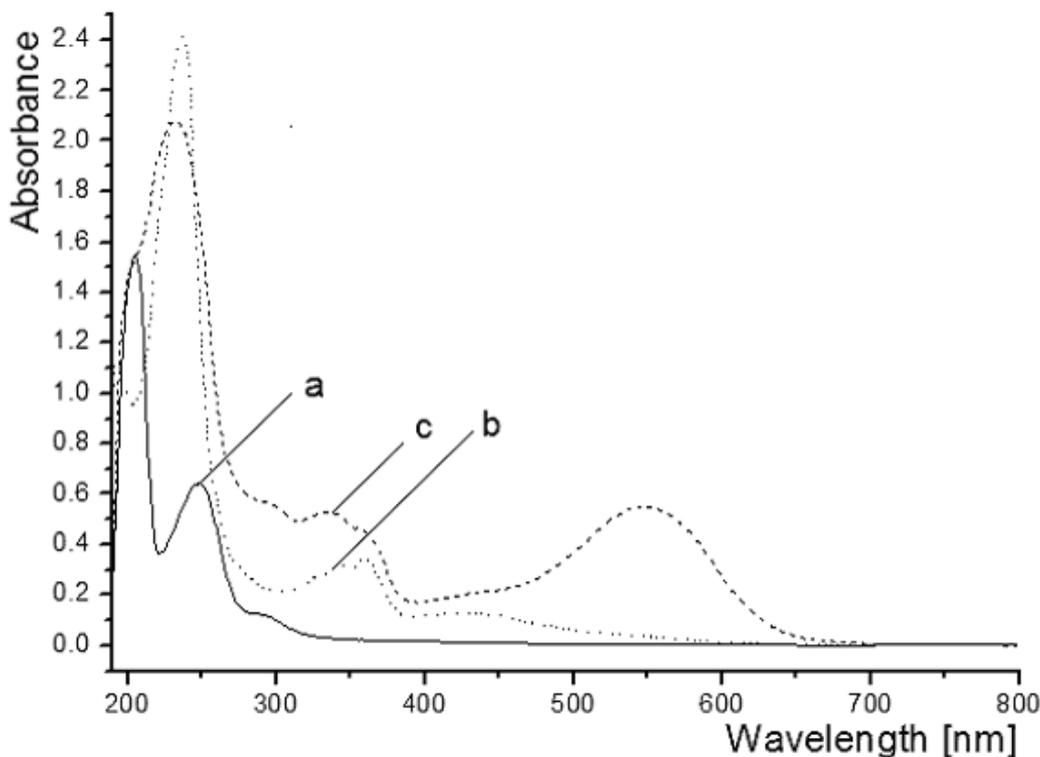

Fig. 2 – UV-VIS spectroscopy analysis; spectra of: **a)** full line-10^{-4} M aqueous solution of DADFM-PAS14 (**1**) **b)** dotted line - 10^{-4} M aqueous solution of H Acid (as sodium salt form) (**3**) **c)** dashed line- 10^{-4} M aqueous solution of compound DADFM-PAS14HA (**5**).

In Fig. 3, the UV-VIS spectra of compound (**6**) versus compounds (**1**) and (**4**) are presented. In this case too, one could notice that the only maximum of absorption (502.2 nm) in the visible domain is presented by compound (**6**).

Both compounds (**5**) and (**6**) were converted to their acidic form, using strong acidic resin type (Amberlite IR120), and their solutions were analysed by UV-VIS spectroscopy. In the case of compound (**5**), its maximum of absorption shows a slightly bathochromic shift, from 547.0 nm to 545.6 nm. The same situation was noticed in the case of compound (**6**), where the bathochromic shift was found among 500.0 nm (the sodium salt of **6**) to 499,2 nm (spectra are not presented). In both situations, no precipitate was noticed.

Compounds **5** (**6**) were further subjected to thermal analysis. For comparison, thermal analysis of compound **1,** then **3** (**4**) and finally of a physical mixture of these two reactants (**1**+**3** (**4**)) were also performed. For these experiments in the physical mixture the reacting **1** and **3** or **1** and **4** compounds were introduced at the molar ratio (1:1) as they were present in the final product **5** and respectively **6.**

The thermograms are shown in Fig. 4 for compound **5** and Fig. 5 for compound **6**. The inspection of Figs. 4 (a-d) and 5(a-c) reveals that the pattern of the three curves corresponding to differential thermal analysis (DTA), derivative thermal gravimetric analysis (DTG) and thermogravimetric analysis (TG) indicates significant differences between the reactants and products, physical mixture of the reactants and the compounds **5** and **6** respectively.

Due to the fact that a maltodextrin with an average molecular weight equal to 1100 was used in the synthesis of **5** and **6**, one could consider that it should be more appropriate to use the concept of materials when referring to dyes **5** and **6**.

In conclusion, two absolutely new dyes were synthesized, using a polysaccharide of low molecular mass as hydrophilic component. This new design will confer to the synthesized dyes a greater solubility, on the entire pH range, and this will result in an extended application field of these dyes, on various substrate types.
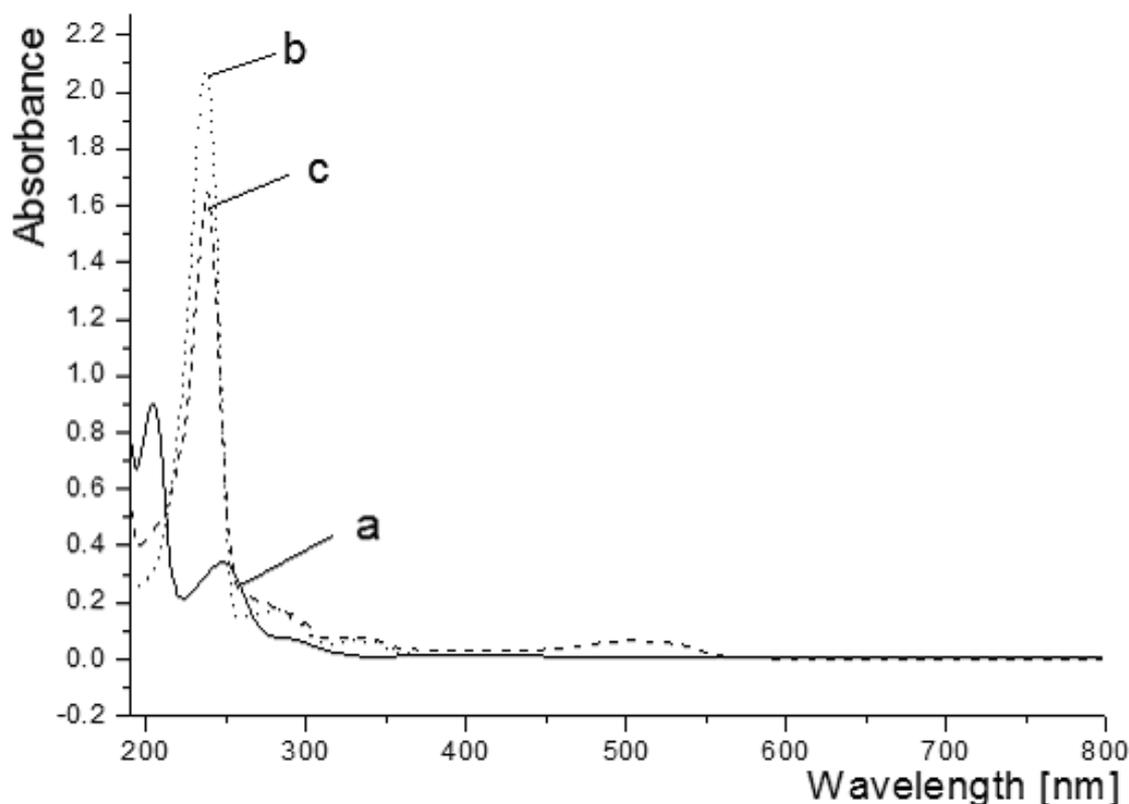

Fig. 3 – UV-VIS spectroscopy analysis; spectra of: **a)** full line-10^{-4} M aqueous solution of DADFM-PAS14 (**1**) **b)** dotted line - 10^{-4} M aqueous solution of R acid (as sodium salt) (**4**) **c)** dashed line- 10^{-4} M aqueous solution of compound DADFM-PAS14RA (**6**).
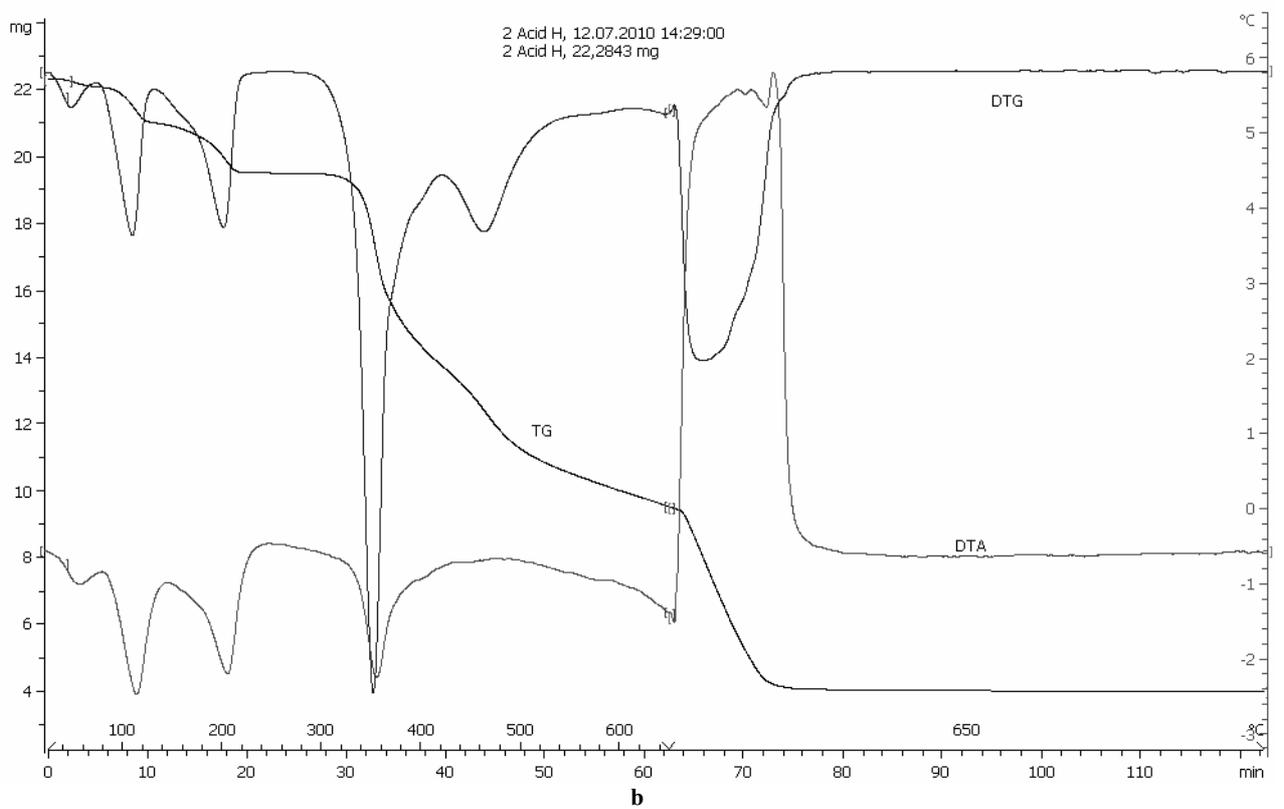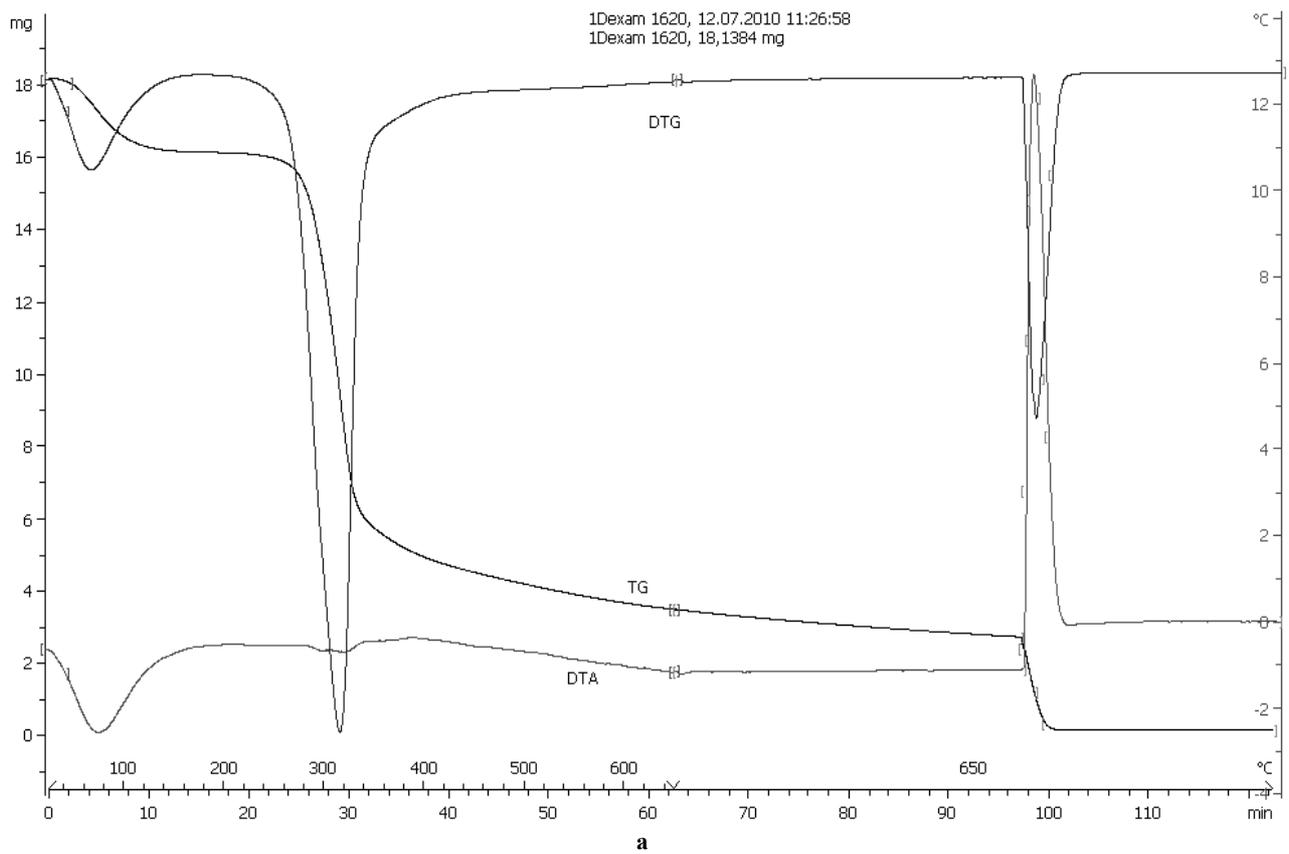
Fig. 4 – Thermal Analysis. Thermal curves (DTA, DTG, TG) of: (**a**) DADFM-PAS14 (**b**) acid H.
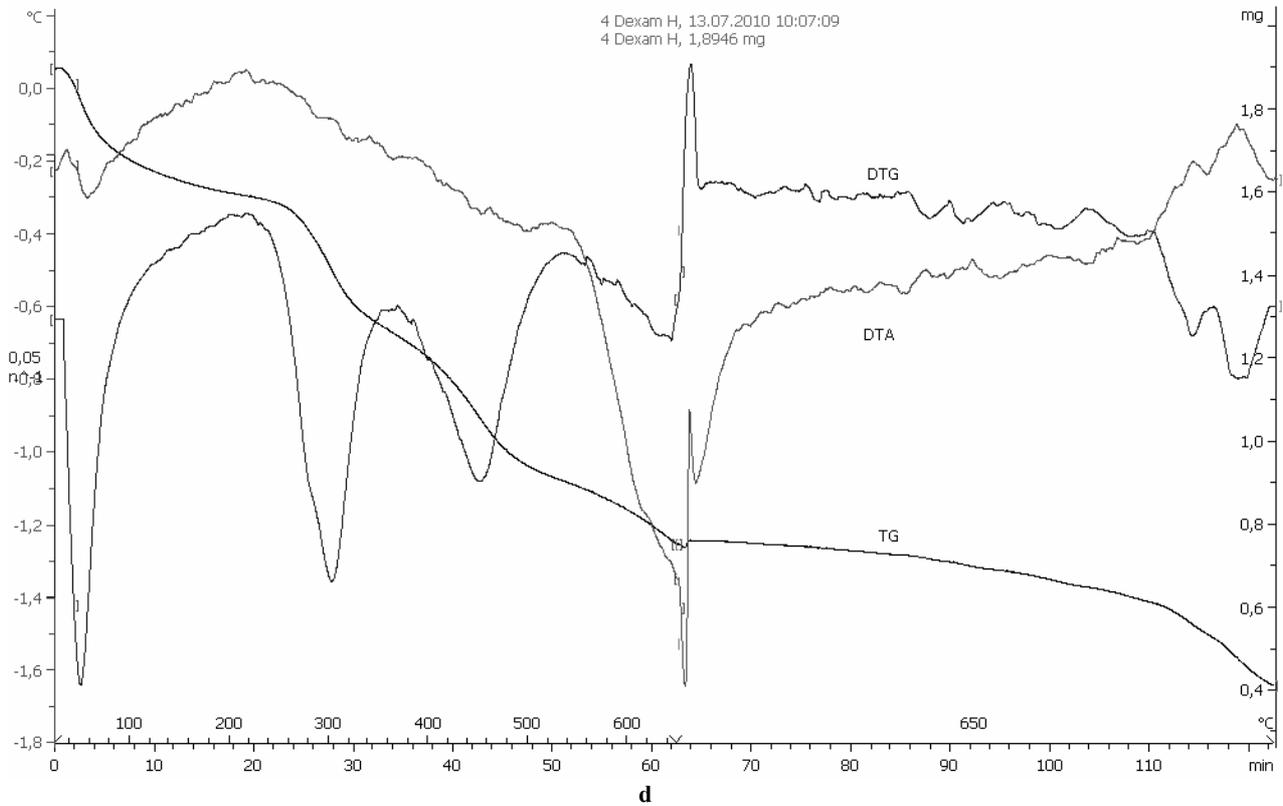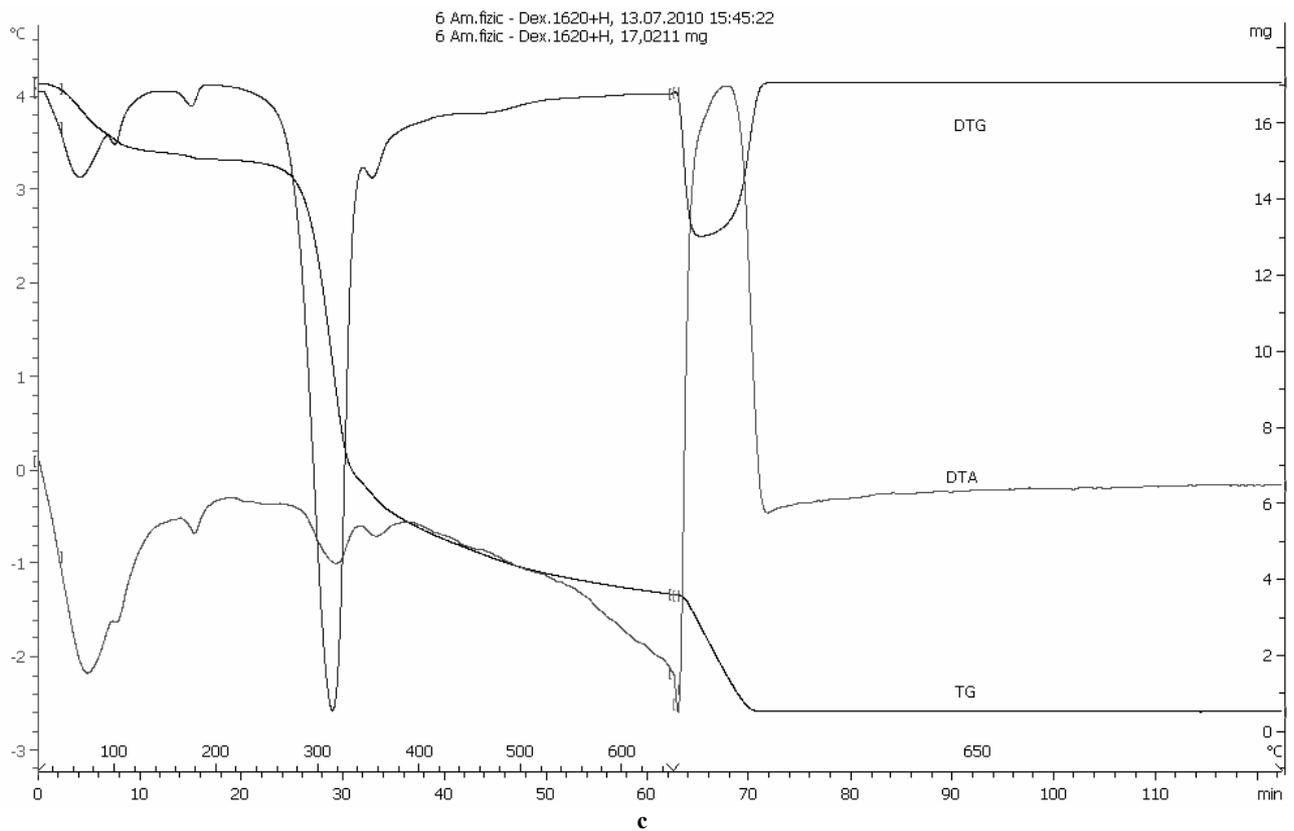
Fig. 4 (continued) – Thermal Analysis. Thermal curves (DTA, DTG, TG) of: (**c**) physical mixture of DADFM-PAS14 and Acid H (1:1 molar ratio) (**d**) compound DADFM-PAS14HA(**5**).
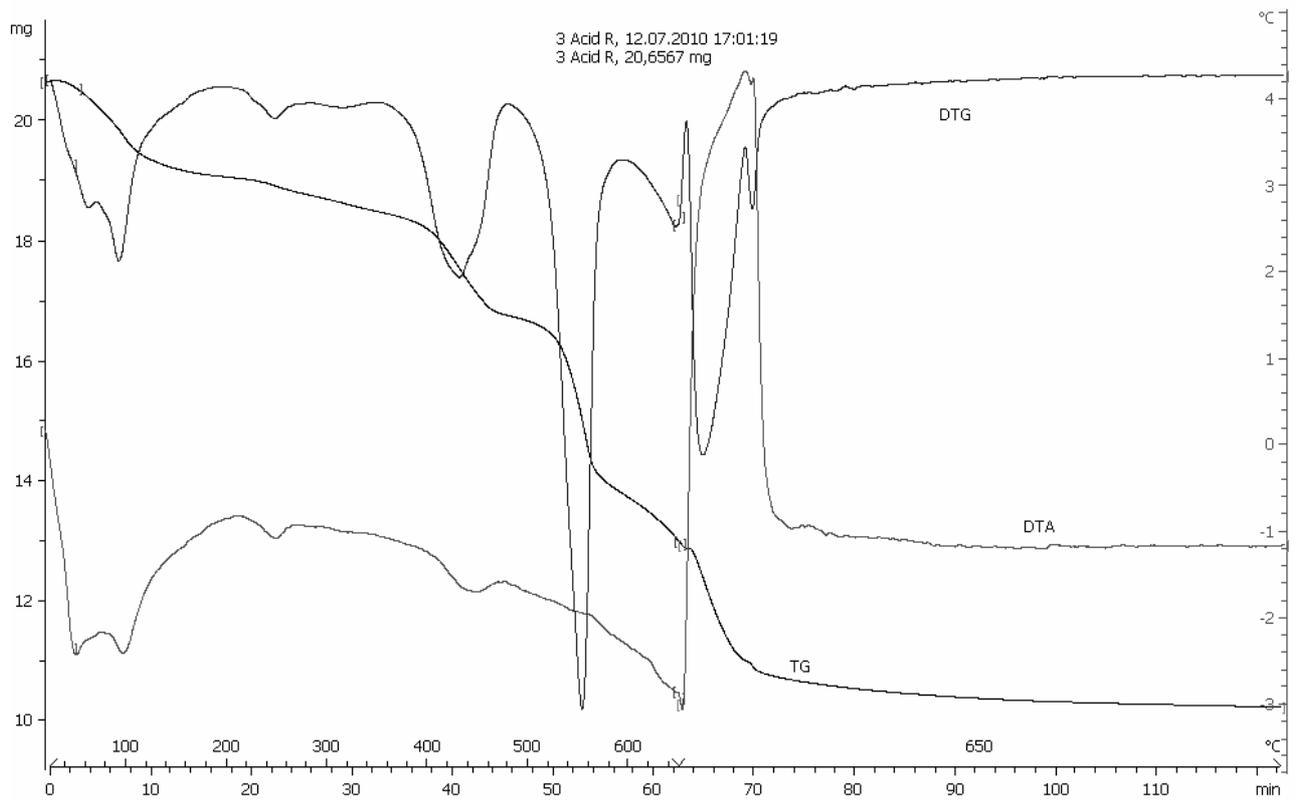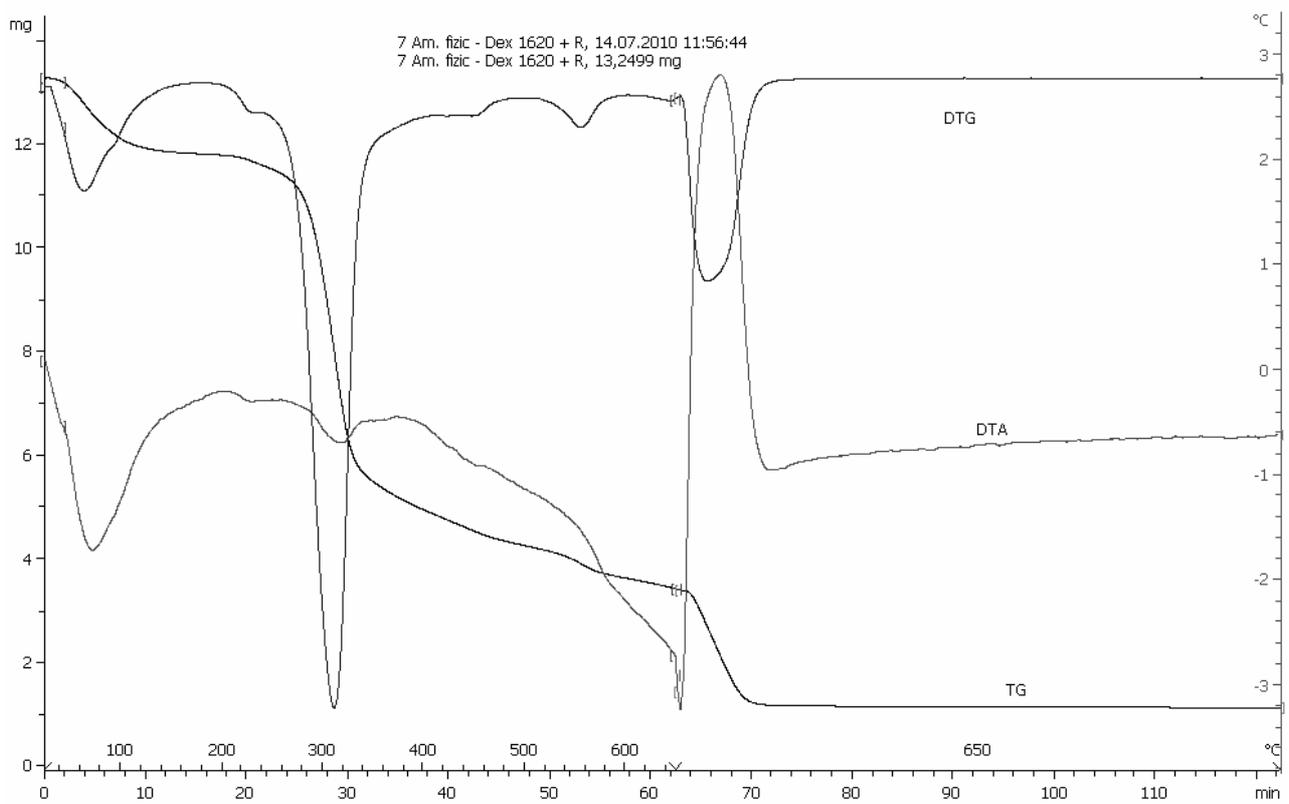
Fig. 5 – Thermal curves (DTA, DTG, TG) of (**a**) acid R (**b**) physical mixture of DADFM-PAS14.
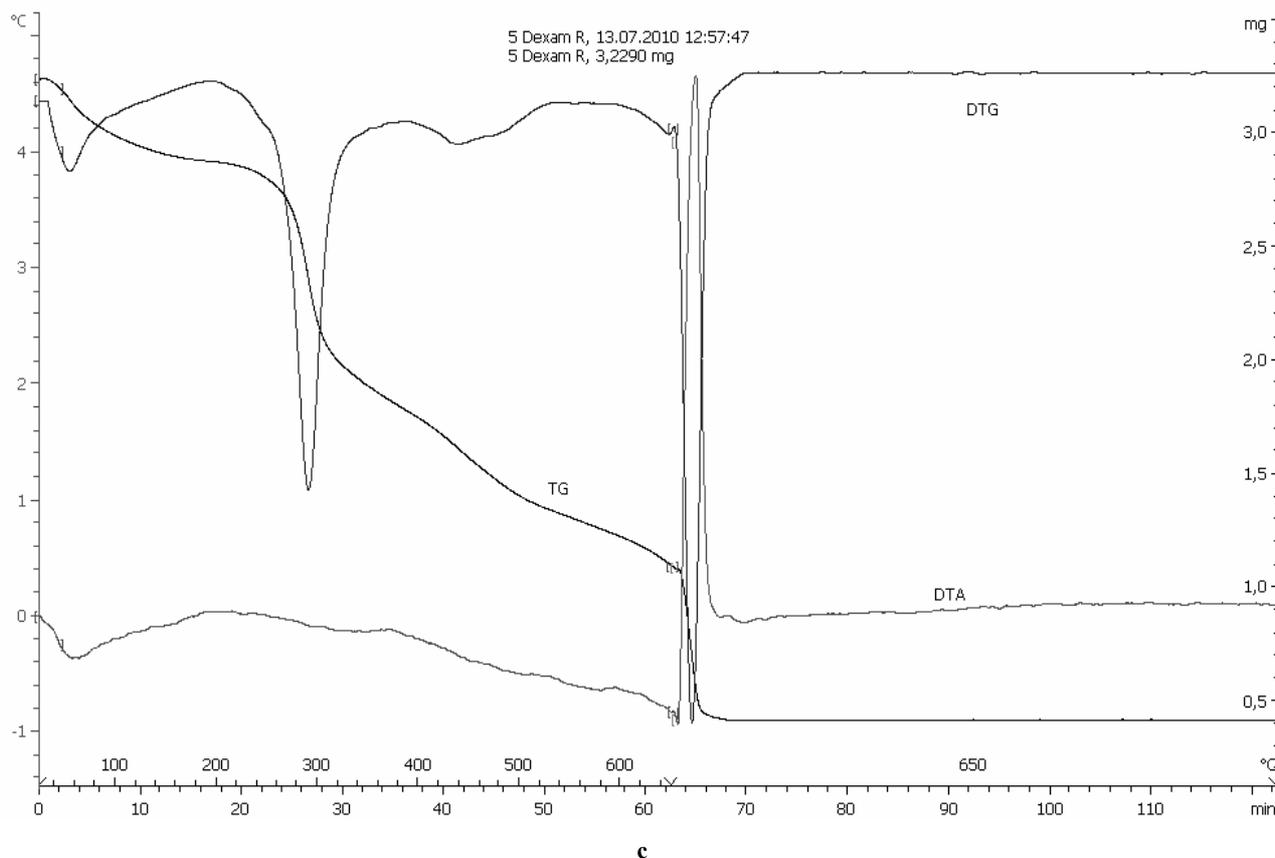
Fig. 5 (continued) – Thermal curves (DTA, DTG, TG) of Acid R (1:1 molar ratio) (**c**) compound DADFM-PAS14RA**(6)**.

EXPERIMENTAL

Materials

Acetic acid (98%), the disodium salts of R acid (technical grade) and H acid (98%) were purchased from Merck (Darmstadt, Germany) and used without further purification. Distilled and deionized water from a Milli-Q water system (Millipore, Bedford, MA, USA) was used for sample solution preparation (UV-VIS and HCIT-MS). Prior to MS analysis, the samples were centrifuged for about 30 min in a Biofuge 13 centrifuge (Heraeus Sepatech, Osterode/Harz, Germany), maltodextrin (Paselli MD14, from AVEBE, Veendam, The Netherlands), 4,4'-Diaminodiphenylmethane, sodium cyanoborohydride, (purchased from Aldrich Chemical Co.,Milwaukee, WI, USA) were used without further purification. Dialysis membrane (molecular weight cut-off (MWCO) 1000) was purchased from Spectrum Europe B.V. (Breda, The Netherlands). The cation-exchange resin (Amberlite IR-120) and the 3A ° molecular sieve were from Sigma (Steinheim, Germany).

Methods

Thermal analysis. Thermal decomposition was carried out using a TGA/ SDTA 851-LF 1100 Mettler instrument. A 30 mg sample aliquot was placed in an alumina crucible of 150 µL. The experiments were conducted under nitrogen atmosphere at a heating rate of 5°C min^{-1}, within 25-650ºC temperature range.

Mass Spectrometry. Mass spectrometry analysis was performed on a High Capacity Ion Trap (HCIT) Ultra PTM mass spectrometer (Bruker Daltonik, Bremen, Germany). The HCT mass spectrometer is interfaced to a PC running the Compass integrated software package under WindowsXP, which includes EsquireControl and Hystar modules for instrument tuning, control and spectrum acquisition, and DataAnalysis software for storing the ion chromatograms and processing the MS data. The samples were infused into MS by online syringe pump electrospray at a constant flow rate of 250 µL/h. Nitrogen at a flow rate of 5 L/min was employed at 250 °C for desolvation and as a nebulizer gas at 7 p.s.i. The instrument was set to operate in the negative or positive ion mode under 3.0 kV ESI potential. For MS analysis the sample was dissolved to a concentration of about 5 pmol/µL, in MeOH/H$_2$O (1:1 v/v).

Infrared Spectroscopy. The infrared spectra were recorded on a JASCO-FT/IR-4200 equipment (single beam) in KBr pallets.

UV-VIS Spectroscopy. The UV spectra were registered on a CECIL CE7200 spectrometer.

Procedures

Synthesis of DADFM-PAS14 (1). The procedure used for the synthesis of DADFM-linked maltodextrin was described in previous works.[12,13] A 50 mL round–bottom flask equipped with magnetic stirrer and refrigerant was loaded with 2.4 g (12 mM) of DADFM, 0.33g (0.3 mM) of Pss14, 0.35 mL of glacial AcOH and 10 mL of H$_2$O. The mixture was stepwise heated for

3 hours up to 75°C. 0.76 g (12 mM) and thereafter, sodium cyanoborohydride was added in small portions over 24 h. After cooling down to room temperature, the reaction mixture was diluted with 100 ml H_2O and the DADFM excess was repeatedly extracted with chloroform. After separation the aqueous phase was removed by vacuum distillation. The obtained product was dissolved in 50 mL water and the polysaccharide was precipitated with 200 mL of absolute ethanol. The precipitate was filtered and repeatedly washed with small portions of absolute ethanol and acetone. The obtained powder was dissolved in 50 mL of deionized water and passed through a strong cationic exchange column (2 cm i.d. × 30 cm length) packed with Amberlite IR-120. The unreacted polysaccharide was washed off with water and reused. The DADFM-PAS14 was desorbed with 10% ammonia solution. The eluate was concentrated under vacuum pressure to 10 mL. This volume was dialyzed against water (dialysis membranes MW/CO=1000). The aqueous solution obtained after dialysis was concentrated by vacuum distillation to yield 310 mg. of white powder of DADFM-PAS14 (Yield = 80%).

Syntesis of (2). In a 20 mL flask equipped with cooling and magnetic stirrer, 2 mL H_2O are added over 0.13 g DADFM-PAS 14 (~10^{-4} mol), 0.007 g $NaNO_2$ (10^{-4} mol) under intense stirring and finally 2 mL HCl 0.1M. The mixture is maintained under stirring at 0-5 °C for about 30 min. A clear yellow suspension is obtained, containing the diazonium salt (2).

Syntesis of (5). In a 50 mL flask equipped with cooling and magnetic stirrer, 3.4 g H acid (10^{-2} mol) are solved in 15 mL sodium carbonate solution (20%). The temperature of the solution is maintained at 3-5 °C and 4 mL solution of the diazonium salt (2) is added dropwise over an hour. After the addition of the diazonium salt is complete, the mixture is maintained under stirring for another hour. During this time, the mixture is allowed to reach room temperature. The crude reaction product was obtained in an overall yield of 84%.

Purification (5). Due to the fact that the coupling component (H acid) was used in a large excess, the separation of the formed dye (5) is performed by size exclusion dialysis. In a dialysis bag (MW/CO=100), 20 mL reaction mixture is loaded. The bag is sealed and dialyzed against water for 24 hours. Then the bag is opened and its contents are TLC inspected in order to check for the presence of H acid. If the solution does not contain any more H acid, the mixture is concentrated under vacuum pressure to dryness. Otherwise the dialysis is continued. The obtained product is dried overnight under vacuum at 40 °C.

The synthesis and purification of compound (6) is similar to compound (5).

DADFM-PAS14 (1). white powder; **IR** (KBr, cm^{-1}): 530.3, 578.5, 759.8, 853.3, 932.4, 1026.9, 1151.3, 1245.89, 1411.6, 1516.7, 1639.2 2929.3, 3386.4; **UV**(λ(nm),$\varepsilon_{max}H_2O$) =205.5, 16000.0, 249.0, 6500.0; **(+)ESI-HCIT-MS**, *m/z*=1497.7 (Glc$_7$Ag); = 1335.6 (Glc$_6$Ag); =1173.5 (Glc$_5$Ag); = 1011.4(Glc$_4$Ag); = 849.3 (Glc$_3$Ag); 687.5 (Glc$_2$Ag); = 525.2 (Glc$_1$Ag), = 413.2 (unresolv.); = 324.4 (Glc$_2$-H_2O), = 304.3, = 274.3 (unresolv.), 144.0 (Glc-H_2O).

DADFM-PAS14HA (5) violet powder; **IR** (KBr, cm^{-1}): 578.5, 670.1, 1044.3, 1202.4, 1378.9, 1506, 1635.3, 3443.3; **UV**(λ(nm), $\varepsilon_{max}H_2O$) = 232.0, 20800, 330.0, 5600.0, 547.0, 5500 (as sodium salt); 230.6, 26500, 545.6, 2400.0 (as free acid).

DADFM-PAS14RA (6) red powder; **IR** (KBr, cm^{-1}): 612.3, 689.4, 854.3, 931.5, 1030.8, 1157.1, 1207.22, 1417.4,

1506.1, 1635.3, 2936.1, 3426. 9; **UV**(λ(nm), $\varepsilon_{max}H_2O$) = 237.6, 16500, 325.8, 800; 502.2, 700 (as sodium salt); 235.0,14500, 330.0, 6600, 499.2, 4570.0 (as free acid).

REFERENCES

1. R. M. Christie, "Colour Chemistry", The Royal Society of Chemistry, Thomas Graham House, Cambridge, Great Britain, 2001, p. 205.
2. E. Coates, *J.S.D.C.*, **1969**, *85*, 355-359.
3. J. Srivastava and P. S. Vankar, *Carbohydr. Res*, **2010**, *345*, 2023-2029.
4. F. Dumoulin, M. Durmus, V. Ahsena and T. Nyokong, *Coord. Chem. Rev.,* **2010**, *254*, 2792-2847.
5. N. Hurduc, R. Enea, I. Moleavin, E. E. Iorgulescu and V. David, *Rev. Roum. Chim.*, **2008**, *53*, 777-785.
6. M. Zhenyi, D. Mingshi and C. Yuhai, *Dyes and Pigments*, **1994**, *25*, 241-248.
7. G. M. Simu, S. G. Muntean, M. E. Grad, S. A. Chicu and E. N. V. Şişu, *Rev. Chim. (Bucharest)*, **2006**, *57*, 1038-1040.
8. G. M. Simu, S. G. Hora, M. E. Grad and E. N. V. Şişu, *Rev. Roum. Chim.*, **2005***, 50*, 113-117.
9. S. Muntean, G. Simu, L. Kurunczi and Z. Szabaday, *Rev. Chim. Bucureşti*, **2009**, *60*, 152-156.
10. S. A. Chicu and G. M. Simu, *Rev. Roum. Chim.*, **2009**, *54*, 659-669.
11. E. Şisu, W. T. Bosker, W. Norde, T. M. Slaghek, J. W. Timmermans, J. Peter Katalinic, M. A. Cohen- Stuart and A. D. Zamfir, *Rapid Commun. Mass Spectrom.*, **2006**, *20*, 209-218.
12. I. Perdivara, E. Şişu, I. Şişu, N. Dinca, K. B. Tomer, M. Przybylski and A. D. Zamfir, *Rapid Commun. Mass Spectrom.*, **2008**, *22*, 773-782.
13. I. Şişu, V. Udrescu, C. Flangea, N. Dinca, S. Tudor, L. Rusnac, A. D. Zamfir and E. Şişu, *Cent. Eur. J. Chem.*, **2009**, *7*, 66-73.
14. A. T. Balaban, M.Banciu and I. I.Pogany, "Aplicaţii ale metodelor fizice în chimia organică", Ed. Ştiinţifică şi Enciclopedică, Bucureşti, 1983.
15. H. Sanielevici and F. Urseanu, "Sinteze de coloranţi azoici", Vol. 1, Ed. Tehnică, Bucureşti, 1987.
16. J. Shore (Ed.), "Colorants and auxiliaries. Organic Chemistry and Application Properties", Vol. 1 – Colorants, 2nd Edition, Bradford, England, 2002.
17. H. Zollinger, "Color a Multidisciplinary Approach", Wiley-VCH, Heidelberg, Berlin, 1998.
18. H. Zollinger, "Color Chemistry: Synthesis, Properties and Applications of Organic Dyes and Pigments", 2nd Edition, VCH, Weinheim, New York, 1998.
19. G. Socrates, "Infrared and Raman characteristic group frequencies: tables and charts", Third Edition, John Wiley&Sons Ltd., Chichister, England, 2004.
20. J. Umemura and S. R. Park, *Vibrational Spectroscopy*, **2005**, *38*, 29-32.
21. M. Snehalatha, C. Ravikumar and I. Hubert-Joe, *Solid State Sciences*, **2009**, *11*, 1275-1282.