Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Polydopamine coated magnetic nanoparticles for isolation and enrichment of estrogenic compounds from surface water samples followed by liquid chromatography-tandem mass spectrometry determination

Anna Laura Capriotti, Chiara Cavaliere, Giorgia La Barbera, Susy Piovesana, Roberto Samperi, Riccardo Zenezini Chiozzi, Aldo Laganà

Table S1 Elemental analysis of Fe_3O_4@pDA magnetic nanoparticles prepared for this work

Component	**Element %**
Nitrogen	2.018465996
Carbon	14.11472034
Hydrogen	1.590201974
Sulphur	<LOD

Table S2 For each analyte, values of pK_a and logarithm of the octanol/water partion coefficient, calculated with the method defined by Ghose and Crippen (ALogP) are provided. Source: ChEMBL database, chemical database of European Bioinformatics Institute, part of European Molecular Biology Laboratory (EMBL-EBI), freely available at https://www.ebi.ac.uk/chembl/

Analyte	**Acidic pK_a**	**ALogP**
E3	10.25	2.87
β-E2	10.27	3.84
α-E2	10.27	3.84
E1	10.25	3.94
β-ZAL	8.08	4.07
β-ZOL	7.83	3.88
α-ZAL	8.08	4.07
α-ZOL	7.83	3.88
ZAN	7.83	3.88
ZEA	7.58	3.44
DAID	7.01	2.38
GLYC	7.03	2.37
GEN	6.51	2.14
COUM	8.25	3.06
EQUOL	9.92	3.03
FORM	6.84	2.37
BIO-A	6.50	2.37

Table S3 Process efficiency (PE)% for the analytes tested. Spiking level was 2 μg L^{-1} for all the analytes but the aflatoxins, for which spiking level was 0.2 μg L^{-1}. The results are the average of two measurements

COMPOUND	**PE(%)**
Aflatoxin B1	74
Aflatoxin B2	74
Aflatoxin G1	65
Aflatoxin G2	61
Sulfadiazine	2
Sulfamerazine	4
Sulfamethoxazole	2
Sulfathiazole	11
Triclosan	0
Tris(2-chloroethyl) phosphate	16
Bisphenol A	28
Tris(chloroisopropyl) phosphate	31
4-Octylphenol	5
4-methylbenzylidene camphor	0
2-phenylbenzimidazole-5-sulfonic acid	27
4-hydroxy-2-methoxybenzophenone-5-sulfonic acid	12
4,4'-dihydroxybenzophenone	73
p-aminobenzoic acid	16
ethyl 4-aminobenzoate	15
2,2',4,4'-tetrahydroxybenzophenone	60
4-hydroxybenzophenone	83
2,4-dihydroxybenzophenone	90
2,2-dihydroxy-4-methoxybenzophenone	82
2-hydroxy-4-methoxy-benzophenone	65
diethylamino hydroxybenzoyl hexyl benzoate	171
2-ethylhexyl 4-(dimethylamino)benzoate	216

1-(4-methoxyphenyl)-3-(4-tert-butylphenyl)propane-1,3-	70
Octocrylene	1
4-Nonylphenol	0
Estriol-3-sulfate	22
17β-estradiol-3-glucuronide-17β-sulfate	15
Estrone-3-glucuronide	21
17β-estradiol-17-glucuronide	29
17β-estradiol-3-glucuronide	21
17β-estradiol-3,17β-disulfate	15
17β-estradiol-17-sulfate	42
17β-estradiol-3-sulfate	45
Estrone-3-sulfate	44
17α-Ethinylestradiol	15
Perfluorobutanesulfonic acid	0
Perfluorohexanoic acid	0
Perfluoroheptanoic acid	0
Perfluorooctanoic acid	0
Perfluorononanoic acid	4
Perfluorooctanesulfonic acid	8
Perfluorodecanoic acid	19
Perfluoroundecanoic acid	7
Perfluorododecanoic acid	12
Perfluorotridecanoic acid	18
Perfluorotetradecanoic	6

Table S4 Instrumental limit of detection (ILOD) and quantitation (ILOQ) expressed as pg injected; Coefficient of determination (R^2) of standard calibration and matrix-matched calibration; RSD of slope and intercept of matrix-matched calibration curve

Analyte	**ILOD** (pg inj)	**ILOQ** (pg inj)	**R^2** Standard calibration	**R^2** Matrix-matched calibration	**RSD** Matrix-matched slope	**RSD** Matrix-matched intercept
E3	5	20	0.9992	0.9960	6	6
β-E2	6	20	0.9998	0.9990	3	3
α-E2	12	40	0.9998	0.9999	1	1
E1	1	3	0.9989	0.9971	5	5
β-ZAL	6	20	0.9996	0.9951	6	6
β-ZOL	6	20	0.9994	0.9946	9	9
α-ZAL	6	20	0.9997	0.9963	5	5
α-ZOL	6	20	0.9997	0.9962	6	6
ZAN	1	4	0.9993	0.9920	7	7
ZEA	0.2	0.5	0.9998	0.9998	1	1
DAID	0.3	1.0	0.9984	0.9976	9	4
GLYC	3	10	0.9996	0.9985	7	3
GEN	3	10	0.9997	0.9855	13	8
COUM	3	10	0.9992	0.9991	5	3
EQUOL	3	10	0.9997	0.9975	8	4
FORM	0.6	2.0	0.9994	0.9950	12	6
BIO-A	0.2	0.5	0.9991	0.9973	9	5

Table S5 Pearson correlation coefficients relative to apparent recoveries (reported in Table 4) obtained at three spiking levels: 0.2, 1 and 2 µg L^{-1} (levels 1, 2, 3, respectively) of estrogens and mycoestrogens, and at 0.1, 0.5 and 1 µg L^{-1} (levels 1, 2, 3, respectively) of phytoestrogens

	Level 1	**Level 2**	**Level 3**
Level 1	-	0.812256	0.776945
Level 2	0.812256	-	0.928531
Level 3	0.776945	0.928531	-

Table S6 Stability and reproducibility ANOVA tests. Stability test was performed during 4 weeks (t0, t1, t2, t3, and t4) and three replicates per time. The critical value for one-way test (P=0.05) was 3.478. Reproducibility test was performed on three batch and three replicates per batch. The critical value for two-way test (P=0.05) was 7.260

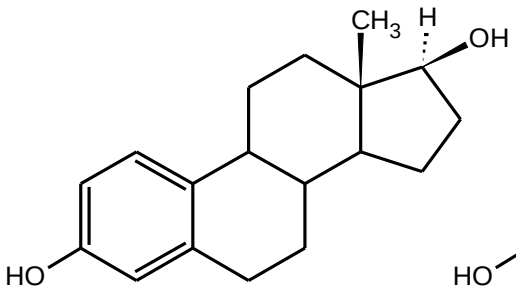
	Stability			**Reproducibility**		
	Mean square			**Mean square**		
Analyte	**Wt[a]**	**Bt[b]**	**F**	**Wb[c]**	**Bb[d]**	**F**
E3	8.04	24.28	3.020	20.64	86.50	4.191
β-E2	13.39	37.89	2.836	32.30	82.07	2.541
α-E2	20.16	47.78	2.378	35.33	172.76	4.890
E1	12.51	46.29	3.049	21.64	133.50	6.169
β-ZAL	3.81	9.98	2.621	9.54	48.72	5.107
β-ZOL	7.73	22.88	2.967	16.83	89.52	5.319
α-ZAL	4.13	9.95	2.416	13.07	20.32	1.555
α-ZOL	2.31	7.65	3.311	33.30	96.20	2.919
ZAN	5.98	13.63	2.273	23.53	140.09	5.954
ZEA	2.89	7.69	2.662	9.84	63.20	6.423
DAID	15.10	39.41	2.617	20.64	98.27	4.761
GLYC	16.58	52.72	3.187	21.44	80.74	3.766
GEN	12.81	30.23	2.363	13.82	35.97	2.603
COUM	21.53	66.64	3.101	16.83	57.49	3.416
EQUOL	5.61	17.95	3.202	18.18	65.50	3.603
FORM	0.95	2.96	3.128	20.28	40.54	1.999
BIO-A	10.09	25.02	2.482	21.08	58.63	2.786

[a]within time; [b]between time; [c]within batch; [d]between batch.

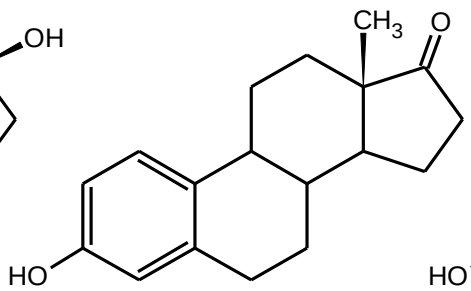
Table S7 Process efficiency (PE, %) and RSDs (*n*=3) obtained analyzing 5 mL pasteurized whole milk samples spiked with 2 µg L^{-1} of estrogens and mycoestrogens and 1 µg L^{-1} of phytoestrogens

Analyte	PE (%) (RSD)
E3	29 (9)
β-E2	45 (5)
α-E2	36 (7)
E1	43 (1)
β-ZAL	27 (4)
β-ZOL	41 (7)
α-ZAL	37 (2)
α-ZOL	52 (3)
ZAN	43 (5)
ZEA	48 (2)
DAID	58 (10)
GLYC	48 (13)
GEN	38 (5)
COUM	54 (2)
EQUOL	51 (15)
FORM	63 (7)
BIO-A	41 (6)

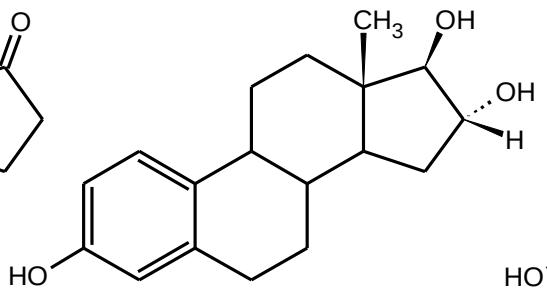
Fig. S1 Structural formula of the investigated analytes



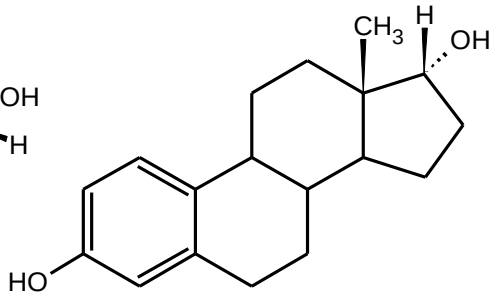
17β–estradiol (β–E2)



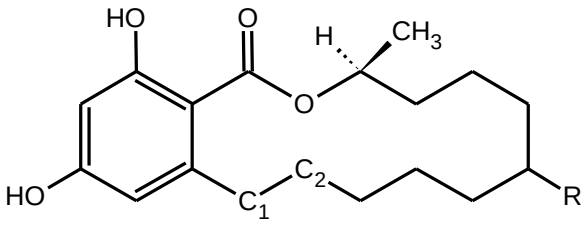
estrone (E1)



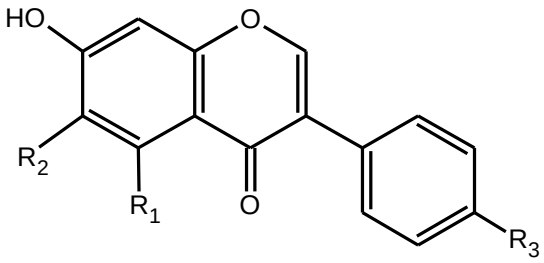
estriol (E3)



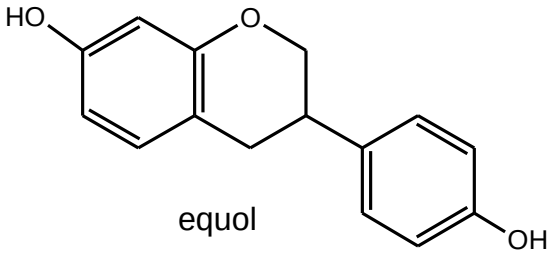
17α–estradiol (α–E2)



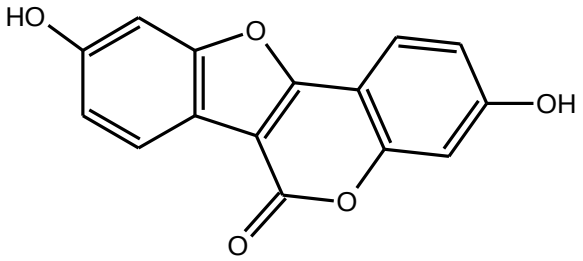
		R	C_1-C_2
zearalenone	ZEA	=O	C=C
α-zearalenol	α-ZOL	⋯OH	C=C
β-zearalenol	β-ZOL	▬OH	C=C
α-zearalanol	α-ZAL	⋯OH	C—C
β -zearalanol	β -ZAL	▬OH	C—C
zearalanone	ZAN	=O	C—C



Isoflavones		**R_1**	**R_2**	**R_3**
daidzein	DAID	H	H	OH
genistein	GEN	OH	H	OH
glycitein	GLYC	OH	OCH_3	OH
formonetin	FORM	H	H	OCH_3
biochanin A	BIO-A	OH	H	OCH_3

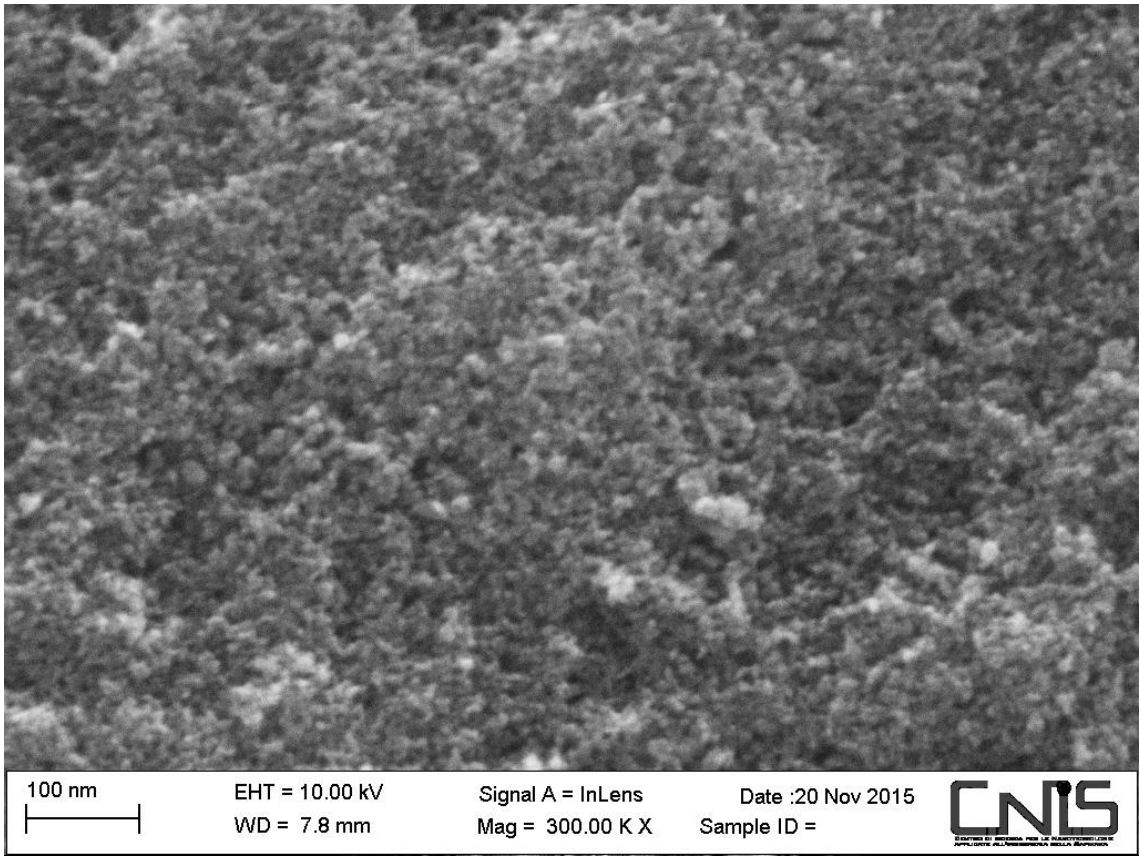


equol



coumestrol (COUM)

Fig. S2 SEM images of Fe_3O_4 magnetic nanoparticles



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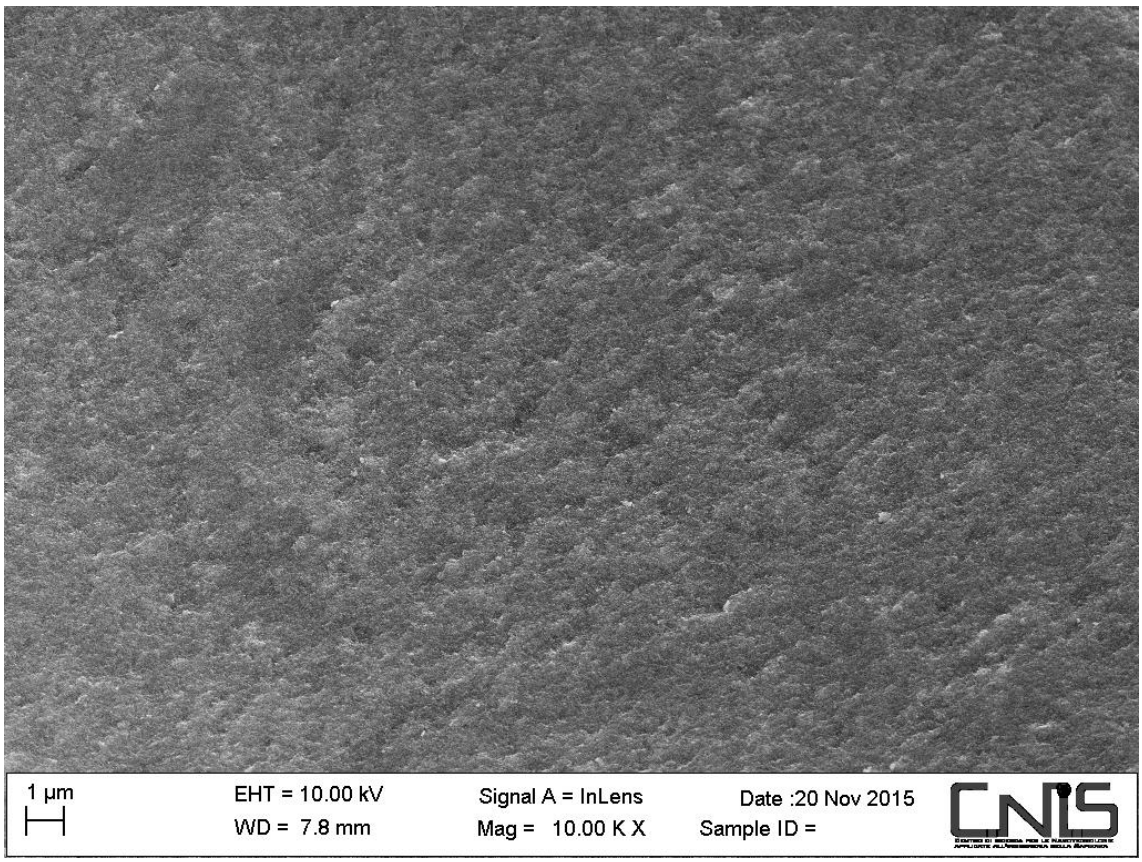
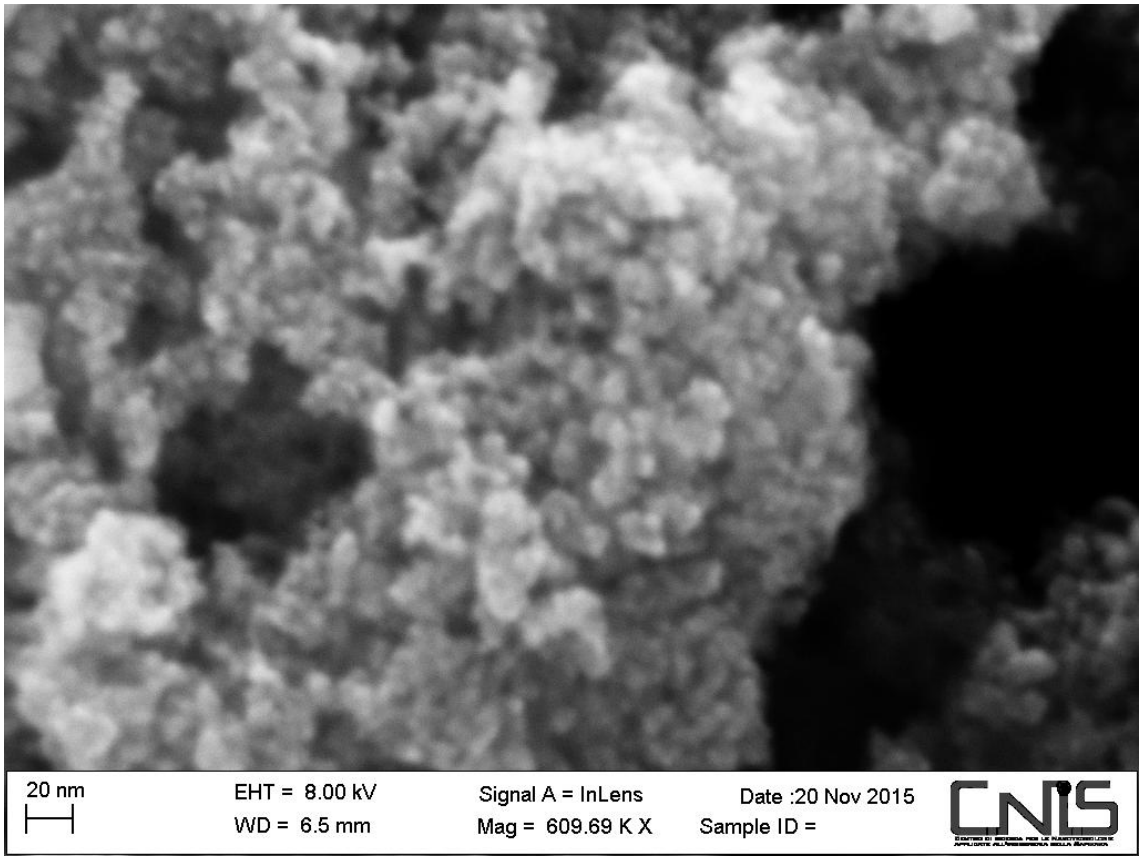

1 µm
EHT = 10.00 kV
WD = 7.8 mm
Signal A = InLens
Mag = 10.00 K X
Date :20 Nov 2015
Sample ID =

Fig. S3 SEM images of Fe_3O_4@pDA magnetic nanoparticles



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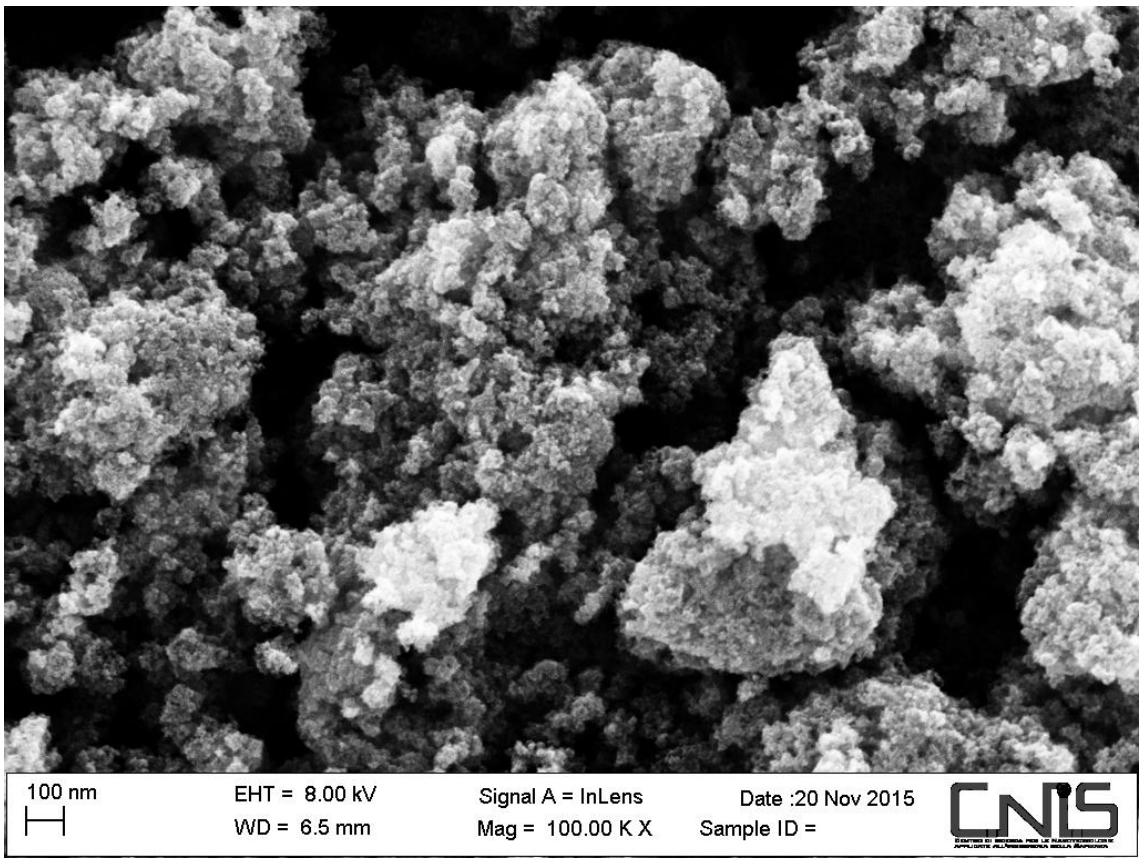
100 nm
EHT = 8.00 kV
WD = 6.5 mm
Signal A = InLens
Mag = 100.00 K X
Date :20 Nov 2015
Sample ID =

Fig. S4 IR spectra of Fe_3O_4 and Fe_3O_4@pDA magnetic nanoparticles