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High Throughput Metabolic Toxicity Screening Using Magnetic Biocolloid Reactors and LC-MS/MS

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Abstract

An inexpensive, high-throughput genotoxicity screening method was developed by using magnetic particles coated with cytosol/microsome/DNA films as biocolloid reactors in 96-well plate format coupled with liquid chromatography-mass spectrometry. Incorporation of both microsomal and cytosolic enzymes in the films provides a broad spectrum of metabolic enzymes representing a range of metabolic pathways for bioactivation of chemicals. Reactive metabolites generated via this process are trapped by covalently binding to DNA in the film. The DNA is then hydrolyzed and nucleobase adducts are collected using filters in the bottom for the 96-well plate for analysis by capillary LC-MS/MS. The magnetic particles facilitate simple and rapid sample preparation and workup. Major DNA adducts from ethylene dibromide, *N*-acetyl-2-aminofluorene and styrene were identified in proof-of-concept studies. Relative formation rates of DNA adducts correlated well with rodent genotoxicity metric TD₅₀ for the three compounds. This method has the potential for high-throughput genotoxicity screening, providing chemical structure information that is complementary to toxicity bioassays.

INTRODUCTION

Humans are exposed to millions of foreign chemicals (xenobiotics) in their lifetimes, including drugs, pesticides, food additives, cosmetics, industrial chemicals and environmental pollutants.¹ Xenobiotics undergo metabolic reactions in the human liver and other tissues that convert them into less toxic, excretable forms. However, some xenobiotics are metabolically *bioactivated* into compounds that react with DNA, proteins and other biomolecules. These processes can result in toxicity, and are often termed metabolic toxicity, or genotoxicity when the target of the reactive metabolite is DNA. Nucleobase adducts formed on DNA are excellent biomarkers for genotoxicity,^{2,3,4} and physiological effects of some DNA adducts are relatively well understood.⁵ Thus, identification of DNA adducts is an important component of toxicity assessment for new drugs or chemicals that will come into contact with humans.⁶

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SUPPORTING INFORMATION AVAILABLE: Detailed procedures of biocolloid reactor film preparation, protein concentration calibration curve and product ion spectra of styrene oxide-DNA adducts are provided. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides sensitive, specific nucleobase adduct detection along with detailed structural information.^{2,4,7,8} We have developed LC-MS/MS methods for toxicity screening of chemicals by coupling colloidal silica bioreactors coated with thin enzyme/DNA films with LC-MS/MS to determine adduct structures and formation rates.⁹ These bioreactor particles feature densely packed DNA/enzyme loadings fabricated by the electrostatic layer-by-layer (LbL) method.¹⁰ They are used to generate metabolites that react with the high surface concentrations of DNA to greatly decrease the time required to obtain DNA adduct samples when reacting enzyme generated metabolite.^{8–12} The first step in chemical screening applications is the metabolic enzyme reaction, in which bioreactors convert test chemicals into metabolites. During this process, DNA in the films captures the reactive molecules as covalent nucleobase adducts. High concentrations of enzymes and DNA in the films ensure a rapid reaction (usually a few minutes) to obtain sufficient products for analyses, as opposed to reaction times of many hours to days when all components are dissolved in solution.⁸ In the second step, nucleobase adducts are released from the particle by hydrolysis and analyzed by LC-MS/MS to obtain adduct structures and formation rates.⁹

We have demonstrated applications of the “biocolloid reactor” method including identification of enzymes responsible for specific metabolic activation,⁹ studies of enzyme inhibition,¹¹ comparison of differences in genotoxic metabolism by rat vs. human enzymes,¹² and metabolic profiling.¹³ However, thin enzyme/DNA film fabrication, including multi-step centrifugation during preparation and product isolation, limits the throughput of these studies. A manual experimental format dictates that only a few reactions can be done and analyzed at a time. In addition, careful control of centrifugation parameters is required to avoid particle aggregation.

Herein, we extend our previously reported high-throughput biocolloid approach for metabolic profiling¹³ to a novel system utilizing magnetic enzyme/DNA biocolloid reactors in a 96-well plate format. The new design achieves high-throughput reactive metabolite screening with LC-MS/MS measurement of DNA adducts. Faster biocolloid reactor particle preparation, enzyme-DNA reaction, and DNA adduct isolation and collection are enabled by magnetic handling in a 96-well plate to facilitate multiplexing (Scheme 1). Chemical structures and relative formation rates of DNA adducts were obtained simultaneously in a high throughput fashion for model carcinogens ethylene dibromide (EDB) and *N*-acetyl-2-aminofluorene (AAF), and the relatively less toxic styrene (Scheme 2). Relative DNA adduct formation rates for these model compounds correlated with the rat liver carcinogenicity metric *toxic dose 50* (TD₅₀).

EXPERIMENTAL SECTION

Reagents and Materials

Carboxylated magnetic particles were from Polysciences (Warrington, PA; ~1 μm diameter; particle concentration 20 mg mL⁻¹). Rat liver microsomes (pooled, Fischer 344) and rat liver cytosol (pooled, Sprague-Dawley) were from BD biosciences (Woburn, MA). All other chemicals were from Sigma-Aldrich.

Film Fabrication

The layer-by-layer (LbL) enzyme-DNA film formation on particles was similar to that in a previous report.¹⁴ Full details are given in the Supporting Information (SI) file. Briefly, polycation poly(diallyldimethylammonium chloride) (PDDA), rat liver microsomes, cytosol and DNA were assembled in alternate steps on the negatively charged magnetic particle surface, in deposition sequences that reverse the charge of deposited material for each subsequent step to facilitate electrostatic adsorption.¹⁵ Steady state adsorption times were 20

min for PDDA and DNA solutions and 30 min for liver microsomes and cytosol while remaining in ice, with washing with Tris buffer after each adsorption step. The magnetic bioreactors were prepared in batches in a 15 mL centrifuge tube (Falcon, BD Biosciences) for later dispensing into a 96-well plate. Notably, multi-step centrifugations were eliminated by the magnetic handling, where particles were trapped onto the centrifuge tube-wall with a lab-built device made from aligned magnets into which the centrifuge tube fits, and then the supernatant liquid was aspirated and discarded. Film architectures of magnetic biocolloid reactors were as follows: PDDA/DNA for reactions with styrene oxide; PDDA/cytosol/PDDA/DNA for reactions with EDB; PDDA/cytosol/PDDA/microsomes/PDDA/DNA for reactions with styrene and AAF.

Sample Workup

Safety note: styrene oxide, ethylene dibromide and *N*-acetyl-2-aminofluorene are suspected carcinogens. All procedures were done under closed hoods while wearing gloves.

(1) Reaction with styrene oxide—A 200 μ L bioreactor dispersion with PDDA/DNA films in 10 mM Tris buffer (pH 7.0) were added to each well in a 96-well plate (500 μ L, Deepwell, Eppendorf). Reactions were started by an addition of 5 μ L of styrene oxide (in acetonitrile, final concentration 5 mM), and terminated by separation of the particles with the reaction matrix by magnetically trapping particles to the side and aspirating the supernatant. Reactions were allowed for various times in minutes at 37 °C, and particles were washed three times with Tris buffer to remove the excess styrene oxide. The particles were dispersed in D.I. water and subjected to neutral thermal hydrolysis in a 90 °C water bath for 1 hr with a well plate cover to minimize the solvent evaporation. Samples were then transferred and filtered through a filtration plate (3 k Da mass cut-off, Pall Life Sciences).

(2) Metabolite-DNA adduct formation—Enzyme reactions with three different substrates were carried in a 96-well reaction plate for different times in triplicate. A final concentration of 200 μ M ethylene dibromide and *N*-acetyl-2-aminofluorene were delivered using acetonitrile (final volume <1%) to a 200 μ L bioreactor dispersion in 10 mM MES buffer (pH 6.5) in each well to initiate the enzyme reactions. Necessary enzyme cofactors were included, i.e. 5 mM glutathione for EDB; 0.5 mM acetyl coenzyme A, 1.6 mM dithiothreitol, 0.5 mM ethylenediaminetetraacetic acid and an NADPH generating system (10 mM glucose 6-phosphate, 4 units of glucose-6-phosphate dehydrogenase, 10 mM MgCl_2 , 0.80 mM NADP^+) for AAF. Reactions were conducted at 37 °C for 1, 3, 5 and 7 min in triplet, and stopped by 1/5 volume of cold acetonitrile with 6% (v/v) formic acid. A 1 hr incubation and 800 μ M styrene were used for the bioactivation of styrene, at the presence of an NADPH regenerating system. After the reaction, bioreactors were washed in the same manner as previously mentioned followed by different hydrolysis methods. Bioreactors reacted with EDB and styrene were subjected to neutral thermal hydrolysis, as previously described for styrene oxide reactions. Bioreactors reacted with AAF were transferred to another plate, and enzymatically hydrolyzed following previous protocol.¹⁴ Briefly, bioreactors in each well were incubated with deoxyribonuclease I (400 unit mg^{-1} of DNA) for 5 hrs, followed by incubation with phosphodiesterase I (0.2 unit mg^{-1} of DNA) and phosphatase, alkaline (1.2 unit mg^{-1} of DNA) for 12 hrs at 37 °C, with a well plate cover. After hydrolysis, two sets of sample were transferred to a filtration plate and spiked with an 88 nM concentration of 7-methylguanosine as an internal standard.

CapLC-MS/MS Analysis

A capillary LC (Waters, Capillary LC-XE, Milford, MA) was used as previously described.¹⁴ A 10 μ L of sample was loaded on a C18 trap column, and flushed at a flow rate of 10 $\mu\text{L min}^{-1}$ with water (with 0.1% formic acid) to eliminate the residual salt and most of the

unmodified bases. After 2 min the adducts were back-flushed to the analytical column and separated using a binary separation gradient composed of ammonium acetate buffer (10 mM, pH 4.5 with 0.1% formic acid) and acetonitrile, with the following acetonitrile composition, 10% for 2 min, 0–25% for 20 min, B; 25% for 2 min and 25–10% for 4 min at a flow rate of 9 $\mu\text{L min}^{-1}$. A 4000 QTRAP (AB Sciex, Foster City, CA) mass spectrometer with Analyst 1.4 software was operated in the positive ion mode. Multiple reactions monitoring (MRM) was conducted at 4500 V ion spray voltage, 40 V declustering potential, 20–40 eV collision energy and 0.15 s dwell time for different mass transitions.

RESULTS

Film Characterization

Similar to previous applications, we used LbL methods to make films on particles containing DNA and cytosol and microsomal enzyme sources.^{9,14,19} However, the use of 1 μm magnetic particles allowed the use of a simple magnetic device to trap particles on the bottoms and sides of a centrifuge tube for washing, solution exchange, and particle isolation instead of using centrifugation. We estimated amounts of biomolecules on the particles (Table 1) based on the amount remaining in solution after the each adsorption step by measuring UV absorbance of the supernatant before and after assembly. The total amount of cytosolic or microsomal protein was obtained using a Bradford assay,²⁰ and a calibration curve was obtained same day using bovine serum albumin (BSA) (SI, Fig. S1). The amount of DNA was obtained based on absorbance at 260 nm (A_{260}).²¹ Film thickness was estimated using total amount of biomolecules in the film divided by particle surface area and density of 1.3 g cm^{-3} .¹⁰

Reaction of DNA with Styrene Oxide

Reactions using magnetic PDDA/DNA biocolloids without enzymes were run simultaneously in the 96-well plate at 37 °C for various times with styrene oxide (SO), the major metabolite of styrene (Scheme 2A).¹⁶ Neutral thermal hydrolysis in the same well plate was then used to selectively release the major N^7 -SO-guanine and N^3 -SO-adenine adducts.^{19,22,23} Samples were transferred to a 96-well filtration plate with a planar magnet placed above (Scheme 1) to pull the magnetic biocolloids up away from the filters at the well bottoms, and allow rapid, efficient vacuum-assisted filtration. Samples were then transferred to an autosampler for LC-MS analysis.

Selected reaction monitoring (SRM) chromatograms in Fig. 1 correspond to SO-guanyl (Fig 1A, mass transition m/z 272>152) and SO-adenyl (Fig 1B, mass transition m/z 256>136) adducts. Both mass transitions reflected a loss of 120 Da, corresponding to styrene oxide. Product ion scans also confirm the major fragment of m/z 272 as 152, [guanine+H]⁺ and the major fragment of m/z 256 as 136, [adenine+H]⁺ (SI, Fig. S2). Reasonable assignments of these adducts are βN^7 -SO-guanine and αN^3 -SO-adenine, which have the largest formation rates of all styrene oxide adducts.²³ Relative formation rates obtained by plotting the area ratio (analyte/internal standard) against reaction time (Fig. 1C) were 0.29 min^{-1} for βN^7 -SO-guanine and 0.15 min^{-1} αN^3 -SO-adenine. The two-fold higher formation rate of βN^7 -SO-guanine compared to αN^3 -SO-adenine is consistent with previous reports of ~3-fold larger amounts of βN^7 -SO-guanine than αN^3 -SO-adenine.²³

Enzyme hydrolysis

Compared to neutral thermal hydrolysis, enzyme hydrolysis releases a much wider spectrum of nucleoside adducts including intact nucleosides, but requires longer incubation time and provides a more complex sample matrix. To test the applicability of enzyme hydrolysis to magnetic biocolloid reactors in the 96-well plates, biocolloids with PDDA/DNA films were

enzymatically hydrolyzed for 17 hrs, and the hydrolysate was analyzed by LC-MS/MS. SRM chromatograms in Fig. 2 demonstrate the successful release of the four native DNA nucleosides featuring a signature neutral loss of sugar moiety (116 Da).

Reactive Metabolite Screening with Bioactivation

Three sets of different enzyme reactions were designed in the same 96-well plate using magnetic biocolloid reactors with different films. Reaction of metabolites of the model compounds, ethylene dibromide, *N*-acetyl-2-aminofluorene and styrene,²⁴ with DNA were investigated simultaneously.

(1) Bioactivation of ethylene dibromide—EDB is a dihaloalkane animal carcinogen used in industry and agriculture.¹⁷ It is bioactivated by cytosolic glutathione *S*-transferase (GST) to form half-mustard reactive intermediate(s) that subsequently attack guanine to form *S*-[2-(*N*⁷-guanyl)ethyl]glutathione as a major DNA adduct (Scheme 2B).^{17,25,26} Biocolloids with PDDA/cytosol/PDDA/DNA films were reacted with EDB in the presence of glutathione, followed by neutral thermal hydrolysis to release adducts. SRM analysis monitoring mass transition m/z 485>356 (Fig. 3A) confirmed the formation of *S*-[2-(*N*⁷-guanyl)ethyl]glutathione. Product ion scan of m/z 485 (Fig. 3B) shows a fragmentation pattern with a m/z 356 as the major product ion, corresponding to loss of a pyroglutamic acid (129 Da), and a m/z 177 correlating to a glutathione residue, as previously proposed.²⁷ When cofactor glutathione was omitted in the reaction, the DNA adducts were not detectable (data not shown), suggesting the necessity of GSH for the catalytic function of cytosolic GST enzymes. Similarly, no adducts were observed for incubations using PDDA/RLM/PDDA/DNA films with GSH alone or with GSH and NADPH. This is consistent with the previous finding that cytosolic GSTs, but not microsomal enzymes, bioactivate EDB into DNA-reactive metabolites.²⁸

(2) Bioactivation of *N*-Acetyl-2-aminofluorene—AAF, a probable human carcinogen, has been used as a model to study metabolic activation and DNA adduct formation, and to validate genotoxicity bioassays.¹⁸ The major bioactivation route for DNA damage by AAF is via *N*-hydroxylated metabolites, mainly catalyzed by cyt P450 1A2, followed by formation of acetylated esters by *N*-acetyltransferase and sulfonated esters via sulfotransferase.¹⁸ These esters break down into nitrenium intermediates that react primarily at C8-guanine site (Scheme 2C).¹⁸ AAF was reacted with magnetic PDDA/cytosol/PDDA/microsome/PDDA/DNA biocolloids using an NADPH regenerating system and acetyl coenzyme A in the same 96-well plate. After reaction, adducted nucleosides were enzymatically released from the particles. A major DNA adduct with mass transition 447>331 (Fig. 3D) was observed, and is most likely *N*-(deoxyguanosin-8-yl)-2-aminofluorene (C8-AF-dGuo) losing a deoxyribose (116 Da), as the C8-guanyl site is a major target of the nitrenium ion.²⁹ A product ion chromatogram in Fig. 3E illustrates that m/z 331 is the major adduct, likely generated from deglycosylation. The second most intense fragment ion (m/z 207), a confirmatory product of C8-guanyl adduct, probably results from cleavage of N7-C8 and C4-N9 bonds of the guanine base (fragmentation pattern in insert of Fig. 3E). This fragmentation mechanism is shared by many C8-guanyl aromatic amine and heterocyclic aromatic amine adducts.^{30,31} The same mass transition was not observed when bioreactors were incubated with AAF only (data not shown), indicating bioactivation was necessary for the formation of C8-AF-dGuo.

(3) Bioactivation of styrene—Styrene is a less genotoxic compared to EDB and AAF, based on liver carcinogenicity metric TD₅₀ (chronic dose mg/kg of body weight per day inducing liver tumors in half of test animal population at end of standard life span).²⁴ When biocolloids with PDDA/cytosol/PDDA/microsome/PDDA/DNA were incubated with 200

μM styrene using an NADPH regenerating system, no DNA adduct was detected within 10 min at the same conditions as used for AAF, indicating much slower bioactivation and DNA adduct formation compared to EDB and AAF. When biocolloids were reacted with 800 μM of styrene for 1 hr, N^7 -guanyl and N^3 -adenyl adducts were observed in SRM chromatograms similar to Fig 1A and 1B, (not shown). The relative formation rates of different adducts were obtained from the slope of the area ratio (adduct/internal standard) versus time (Figures 3C and 3F).

Formation rates of different DNA adducts of the three test compounds were normalized to substrate concentration, and plotted against the inverse of TD_{50} (Fig. 4). Comparable normalized formation rates, i.e. $15.6 (\text{mM of substrate} \cdot \text{min})^{-1}$ for the major EDB adduct and 13.7 for the AAF adduct, correlate well with the similar EDB and AAF TD_{50} values.²⁴ A much smaller DNA adduct formation rate, 0.11, was observed, consistent with a lower $1/\text{TD}_{50}$ value of styrene.

DISCUSSION

Reproducibility of film fabrication and good particle dispersability are crucial for quantitative studies of metabolite-DNA adduct formation using the biocolloid reactor method. Magnetic handling eliminates centrifugation during film preparation required for non-magnetic particles, and minimizes the chance of co-precipitation of DNA and proteins, thus resulting in a better film reproducibility. The time for biocolloid preparation is cut at least in half by magnetic handling compared with multi-step centrifugation and dispersion. In addition, magnetic handling facilitates in situ characterization of the film during fabrication. Results in Table 1 demonstrated reproducible composition for DNA and protein in different film configurations. Similar DNA content in different films facilitates quantitative comparison of DNA adducts when using these films in different enzyme reactions.

For proof of concept, a series of reactions varying in reaction time were done simultaneously in a 96-well plate using magnetic biocolloid reactors with a reactive metabolite, styrene oxide. The two major adducts observed, i.e. N^7 -guanyl and N^3 -adenyl styrene oxide adducts (Fig. 1A and 1B), are consistent with our previous findings using silica particles⁹ and other studies using solution reactions.²³

The capability of detecting various adducts was enhanced by combined use of neutral and enzyme hydrolysis methods. Because of the labile glycosidic bonds of N^7 -guanyl and N^3 -adenyl adducts,⁴ neutral thermal hydrolysis features a fast and selective release of these adducts, which results in a relatively clean sample. Using neutral thermal hydrolysis, we observed the previously reported major DNA adduct, *S*-[2-(N^7 -guanyl)ethyl]glutathione,^{17, 25,26} after metabolic activation of ethylene dibromide (Fig. 3A and 3B).

Enzyme hydrolysis, on the contrary, is applicable to adducts generated from nearly all types of alkylation mechanisms. However, 5 to 20-hour incubations are usually required, and the hydrolysate is more complex than that of thermal hydrolysis. The feasibility of hydrolyzing DNA films enzymatically in 96-well plates was first shown using magnetic biocolloid reactors with intact PDDA/DNA films, indicating the successful release of nucleosides from DNA films on magnetic particles (Fig. 2). Generally, the enzyme hydrolysate requires further purification, such as solid phase extraction, because DNA adducts generated from bioactivation range from 1 adduct per 10^4 to 10^8 unmodified DNA bases.³² A number of steps in the experimental design helped avoid extensive sample purification, including magnetic separation of the biocolloids from the reaction mixtures, downstream low mass cut-off (3k) filtration, and sample pre-concentration using an on-line LC trap column.

Having DNA films on particles provides faster reaction rates due to very high nucleobase concentrations at the site where metabolites are formed, i.e. on the biocolloid reactor, and avoids time-consuming, labor intensive DNA precipitation and isolation needed for sample workup with solution reactions. Observation of a major DNA adduct from bioactivation of AAF, C8-AF-dGuo (Figures 3D and 3E), correlates well with other studies,^{18,29} and demonstrates that this methodology is able to identify a particular adduct from enzyme hydrolysates resulting from a relatively short, simple, multiplexed sample workup.

The high throughput reaction design allows simultaneous metabolic toxicity studies of multiple compounds and controls under different conditions, e.g. different concentrations, metabolic activation pathways, and reaction times (Scheme 1). In this work, representative compounds featuring different degrees of genotoxicity, i.e. EDB, AAF and styrene, were investigated simultaneously. The relative formation rates of different DNA adduct (Table 2) were correlated to carcinogenic potencies as shown by TD₅₀ correlations (Fig. 4).

Magnetic handling and reaction design in the 96-well plate format enabled reaction chemistry and hydrolysis to be done in the same plate without transferring the samples, provided that a common hydrolysis method is used. Agitation can be accomplished in all reaction wells by switching the direction of the field of a magnet on top of the plate, which is of particular importance for short time reactions. During filtration, magnetic bioreactors are suspended by the magnet without settling down to avoid clogging the filter membrane (Scheme 1D). This results in 0.5–1 hr shorter filtration times compared with previously used silica particles.¹³ Taken together, magnetic handling of biocolloid reactor particles in a 96-well plate format facilitates enzyme-DNA reaction, hydrolysis and sample manipulation and improves the throughput of sample preparation by at least 96-fold for reactive metabolite-DNA adduct analysis compared to manual reactions used with silica particle biocolloids.

In summary, we have demonstrated an inexpensive, non-robotic, high-throughput methodology for DNA-reactive metabolite screening using magnetic biocolloid reactors in a 96-well plate coupled with LC-MS/MS. Valuable structure and formation rate information can be obtained in high throughput fashion, and results are complementary to existing genotoxicity bioassays. We are currently testing this methodology with a wider spectrum of chemicals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

LC-MS SRM chromatograms and formation rate plot for styrene oxide DNA adducts using PDDA/DNA bioreactors with 5 mM styrene oxide. (A) βN^7 -SO-guanine with mass transition m/z 272>152 (t_R = 9.54 min), 5 min reaction; (B) αN^3 -SO-adenine with mass transition m/z 256>136 (t_R = 9.12 min), 5 min reaction. (C) Influence of reaction time on βN^7 -SO-guanine (■) and αN^3 -SO-adenine (●), area ratio to internal standard 7-methylguanosine (m/z 298>166).

Figure 2.

LC-MS/MS SRM chromatograms for deoxynucleosides: (A) deoxyadenosine, mass transition m/z 252>136 (t_R = 6.63 min), (B) deoxyguanosine, mass transition m/z 268>152 (t_R = 5.64 min), (C) deoxythymidine, mass transition m/z 243>127 (t_R = 5.79 min), (D) deoxycytidine, mass transition m/z 228>112 (t_R = 4.89 min).

Figure 3.

LC-MS/MS analysis of reactions of magnetic biocolloid reactors with EDB (A, B and C) and AAF (D, E and F). (A) Representative SRM chromatogram with mass transition m/z 485>356 indicating the formation of *S*-[2-(N^7 -guanyl)ethyl]glutathione after 5 min reaction followed by neutral thermal hydrolysis. (B) Product ion spectrum of m/z 485 with inserted fragmentation. (C) Relative formation rate of *S*-[2-(N^7 -guanyl)ethyl]glutathione obtained from area ratio of analyte/internal standard. (D) Representative SRM chromatogram with mass transition m/z 447>331 indicating the formation of C8-AF-dGuo after 5 min reaction followed by enzyme hydrolysis. (E) Product ion spectrum of m/z 447 with inserted fragmentation. (F) Relative formation rate of C8-AF-dGuo from area ratio of analyte/internal standard.

Figure 4.

Comparison of normalized DNA adduct formation rates obtained using magnetic biocolloid reactors and LC-MS/MS vs. the inverse of rodent carcinogenicity metric TD₅₀ (rat).²⁴ (A) Overall formation rate of *N*⁷-SO-guanine and *N*³-SO-adenine from reaction with styrene; (B) *S*-[2-(*N*⁷-uanyl)ethyl]glutathione from enzyme reaction with EDB; (C) C8-AF-dGuo from enzyme reaction with AAF. The normalized formation rate was defined as, (peak area analyte/internal standard) min⁻¹ (mM of substrate)⁻¹.

Scheme 1.

Experimental steps for metabolic toxicity screening using biocolloid reactors in a 96-well plate coupled with LC-MS/MS: (A) enzyme reactions are run; the center 96-well plate illustrates a possible multi-experiment design; (B) while particles are held in the wells by the magnetic plate, solution is replaced with a hydrolysis cocktail; (C) hydrolysis is done; (D) the magnet is moved to the top of the well plate to pull biocolloids away from the filters, and nucleobase/deoxynucleoside adduct samples are filtered into a second 96-well plate; and (E) samples in the second 96-well plate are analyzed by LC-MS/MS.

Scheme 2.

Major metabolic pathways of styrene,¹⁶ ethylene dibromide¹⁷ and *N*-acetyl-2-aminofluorene¹⁸ leading to DNA adduct formation. (Adducts 3, 4 and 7 are presented in nucleobase form released by neutral thermal hydrolysis, and adduct 11 is presented in nucleoside form released by enzyme hydrolysis.)

Table 1Quantitation of biomolecules and film thickness on magnetic particles^a

Composition (/mg of particles)	DNA (μg)	cytosol (μg of protein)	microsomes	Total film thickness (nm)
PDDA/DNA	14.1 $\pm$ 0.3	—	—	9
PDDA/cytosol/PDDA/DNA	30.9 $\pm$ 0.4	22 $\pm$ 2	—	34
PDDA/microsomes/PDDA/cytosol/PDDA/DNA	34.1 $\pm$ 0.4	29 $\pm$ 10	75 $\pm$ 13	89

^aData represent mean $\pm$ SD from 3 replicate samples.