Odor Chemistry of Un-derivatized Human Blood

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Description of Human Blood

Blood is an extremely complex mixture, and the number of different components, which can be detected in its aroma, owes as much to the separating ability and sensitivity of the method used as it does to the sample itself. Blood plasma is a water matrix containing carbohydrates and sugars, lipids and fatty acids, proteins, and nucleotides. The more volatile and primarily odor producing chemicals of blood are large numbers of molecular chains involving 20 amino acids. Amino acids are linked by peptide bonds, a type of covalent bonding, forming proteins. There are short chains of amino acids, peptides and long chains called polypeptides. Amino acids are relatively low molecular weight organic acids, containing an amine (-NH2) group and a carboxylic acid group (-COOH). Amino acids are the basic building blocks of proteins and are mainly responsible for the odor of blood.

Amino acids have always presented a challenge because of significant differences in their chemical structure stretching from non-polar to highly polar. Their low vapor pressure and, in some instances, thermal liability often require synthesis of derivatives that are more volatile and easier to detect and quantify. Detectability has always presented a difficult task for analysts to solve requiring special techniques for gas chromatography, liquid chromatography and in capillary electrophoresis (CE). However with the development of sufficiently sensitive GC detectors, blood odors can now be chromatographed directly without derivatization.

New Analytical Tool for Odor Measurements

A new type of portable gas chromatograph, the zNose™, is able to perform analytical measurements of volatile organic vapors and odors in near real time with part per trillion sensitivity. Because of its picogram sensitivity it is a useful tool for the studies of bio-chemical and metabolic processes involving volatile organics of all kinds. The zNose™ separates and quantifies the organic chemistry of odors through ultra-high speed chromatography in 10 seconds. Using a patented solid-state mass-sensitive detector, picogram sensitivity, universal non-polar selectivity, and electronically variable sensitivity has been achieved. An integrated vapor preconcentrator coupled with the electronically variable detector, allow the instrument to measure vapor concentrations spanning 12 orders of magnitude. The technology has been incorporated into three different instrument types, shown in Figure 1, which are designed for different applications. The high throughput of the Model 7100 laboratory GC (300 plus measurements per day), makes it ideal for auto-samplers while the multi-port Model 7110 zNose™ is designed to automatically monitor multiple vapor streams of chemical processes in real time. The portable Model 4200 handheld zNose™ incorporates a disposable helium tank and allows for onsite odor and ambient air measurements.

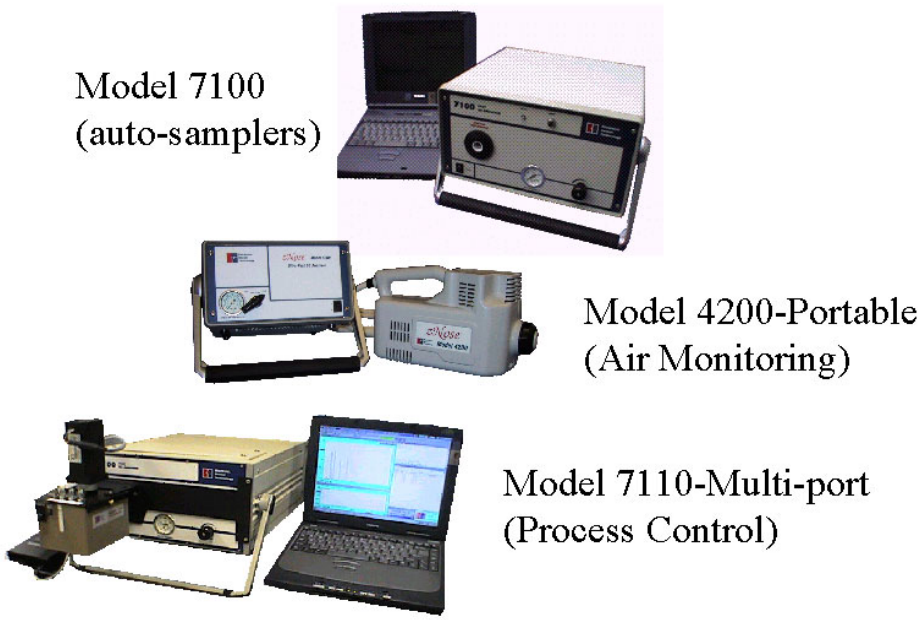

Figure 1- zNose™ technology incorporated into 3 commercial instruments.

Blood Odor Chemistry

The odor chemistry of blood can be estimated by sorting the amino acids by molecular weight. The names and concentration range for each acid among three subject age groups can provide an initial estimate of odor intensities. Other physical properties, such as vapor pressure or boiling point could also be used to estimate the chemical signature of blood odors.

Based upon the properties listed in Table I, an estimated chromatogram response might be as shown in Figure 3.

	Molecular-Weight	Plasma or Serum umole/liter		
Age in Years		0-2	2-10	>10
Glycine	75.07	178-248	117-223	120-553
Alanine	89.09	239-345	137-305	209-695
Serine	105.09	104-158	79-112	67-193
Proline	115.13	141-245	68-148	100-442
Valine	117.15	123-199	128-283	116-315
Threonine	119.12	141-213	42-95	79-246
Cystine	121.16	16-26	23-39	24-71
Taurine	125.14	101-181	57-115	27-168
Ornithine	132.162	39-61	27-86	29-125
Aspartic Acid	133.1	17-21	<20	<24
Isoleucine	131.17	31-47	28-64	35-97
Lysine	146.19	107-163	71-151	82-236
Glutamic Acid	147.13	27-77	23-250	14-192
Glutamine	146.15	623-895	676	413-690
Methionine	149.21	15-21	11-16	6-39
Histidine	155.16	64-92	24-85	31-106
Phenylalanine	165.19	45-65	26-61	37-115
Tryosine	181.19	33-75	31-71	21-87

Figure 2- Common organic chemicals in human blood.

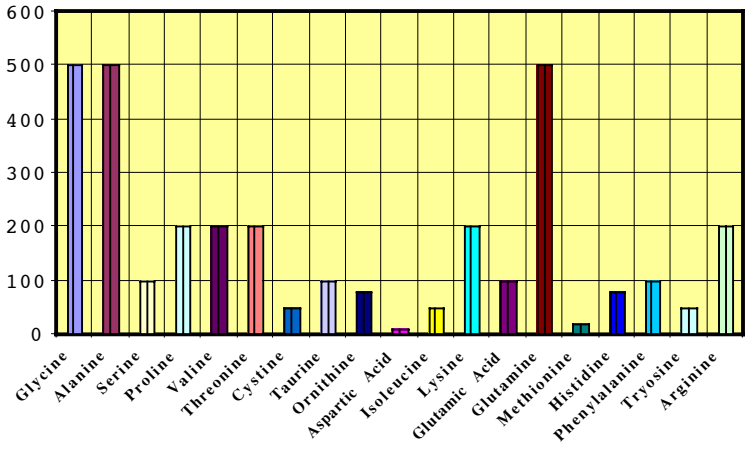

Figure 3- Projected response based upon molecular weight and concentration .

Odor Chemistry

The zNose™ GC measures the concentration of the odor chemicals directly and retention times for each of the chemicals detected are determined by identifying peaks in the GC column flux. Column flux is computed in real time by mathematically performing the time derivative of the detector signal. The result is a chromatogram spanning 10 seconds and representing the rate of adsorption and de-sorption of vapors onto the mass-sensitive detector. Tabulating the retention times together with the individual and total concentration counts (cts) provides a quantitative measure of the chemistry within an odor.

The retention time of any chemical within an odor is referenced to the retention time of a standard odor mixture of linear chain n-alkanes. Shown in Figure 4 is the odor response obtained using methanol spiked with C6 through C14 alkanes. Retention times of unknown peaks when referenced to the n-alkanes are called Kovats indices and allow the use of retention time libraries to aid in future identifications. Indices for the n-alkanes display the 4-digit notation of Kovates indices e.g.c14=1400.

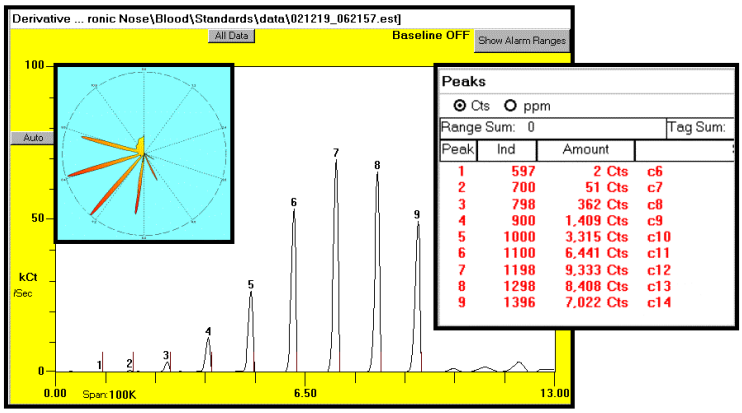

Figure 4- Retention time calibration using n-alkane response C6 to C14. All relative retention times are called Kovats indices.

Kovats Indices of Amino Acids

The retention times and Kovats indices of amino acids were obtained by spiking a septa-sealed vial with a mixture of amino acids of known concentration and measuring the vial headspace odor chemistry with a zNose™. Ten microliters of an amino acid mixture was injected into a 40 milliliter septa sealed vial and thermostated at 37°C. The standard mixture contained 1.26 micromoles of cystine and 2.50 micromoles per milliliter of L-lysine,L-histidine, L-arginine, L-aspartic acid, L-threonine, L-serine, L-glutamic acid, L-proline, glycine, L-alanine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tryosine, and L-phenyl alanine.

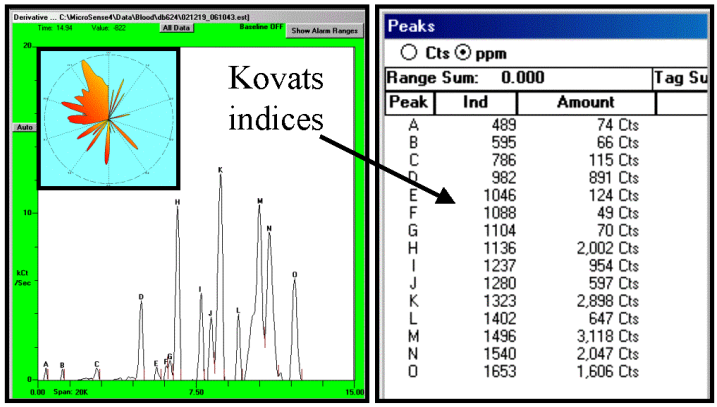

Figure 5- The derivative of odor intensity is a chromatogram used to determine chemical retention times. An analysis of odor from amino acid standards showing Kovats indices and odor concentration in counts.

The odor chemistry obtained by directly sampling 15 milliliters of headspace vapor is shown in Figure 5. Each peak corresponds to a different amino acid and the Kovats indices of all peaks detected spanned 489 to 1653 relative to the retention time of the n-alkanes. In this experiment a room temperature sample needle prevented higher molecular weight compounds, such as fatty acids, from being collected. However, using direct sampling with a heated 200°C inlet enables the zNose™ to detect even these compounds with Kovats indices up to approximately 2600.

Blood Odor Measurements

The solid-state detector directly measured odor intensity of blood samples vs elution time from a GC column, which was temperature programmed from 40°C to 160°C at 10°C per second. Sensitivity was controlled by the temperature of the detector (20°C) and the amount of the vapor sampled (15 milliliter). Extracting and preconcentrating a 15-milliliter vapor sample from the vial measured the concentration of chemical vapors from one drop of blood to be measured in less than one minute. Background odors from ambient air were not a factor at these odor concentrations.

The odor chemistry measurement results are compared in three vertically offset chromatogram traces (Figure 6) corresponding to two blood samples and the headspace vapors from a vial containing the amino acid standards. With few exceptions, the odor chemistry of un-derivatized blood matches that of the amino acid standards and from this experiment it is concluded that the major odor producing chemicals in blood are in fact the amino acids.

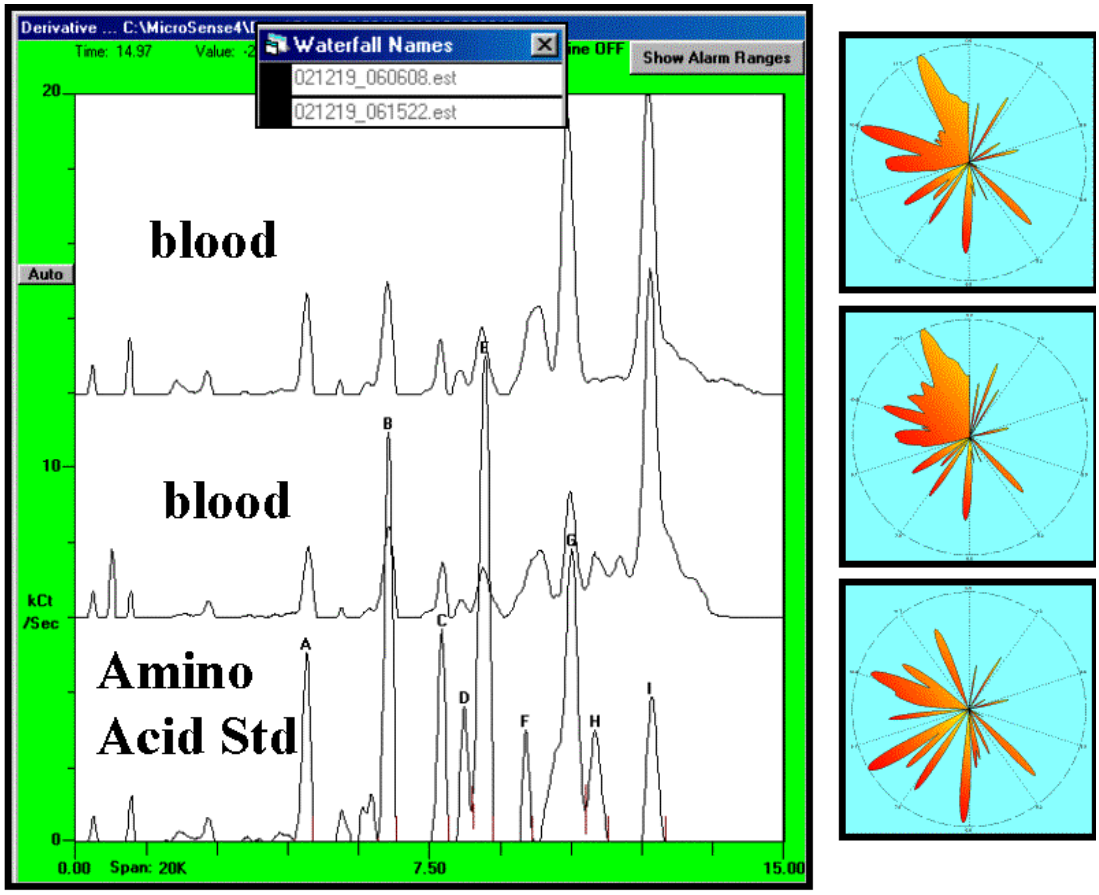

Figure 6- Odor chemistry of blood compared with odor from amino acid standards

Olfactory images, called VaporPrints™, are high-resolution 2-dimensional images based upon the relative concentrations of the individual chemicals making up an odor. These images form a polar display of the odor intensity (radial direction = sensor signal) or odor flux (radial direction = derivative of sensor signal). Retention time (volatility) or Kovats indices is represented by the angular variable with 0 and maximum retention time at the 12 o'clock position of the image. Their characteristic shapes based upon the odor's unique chemistry can easily be recognized in this format. In effect, the olfactory image transforms the human olfactory response into a visual response. Humans and computers are well suited to the analysis and recognition of visual patterns. Computer processing of olfactory images quantifies the individual chemicals and allows the aggregate odor response to be recognized relative to known odors and chemical vapor standards.

Summary of Results

A new type of electronic nose based upon ultra high-speed gas chromatography and a new solid state GC detector now allows the chemistry of odors to be qluantified in near real time with high precision, accuracy, and part per trillion sensitivity. Odors from blood samples and amino acid standards were characterized and compared using chromatograms and visual olfactory images based upon chemical measurements. The major volatile chemicals were found to be the simple amino acids, the building blocks of proteins in the blood. The sensitivity of the instrument allowed odor chemical concentrations at part per trillion to be made without derivatization.

Because the electronic nose is based upon the science of gas chromatography, odor measurements can be easily confirmed and validated by independent laboratory measurements taken on quality control samples. The ability to rapidly perform analytical measurements on blood and other biological samples in real time provides researchers with a cost effective new tool for monitoring volatile organic compounds associated with biological samples such as blood and urine.