A novel approach for the colorimetric detection of creatinine in a paper/polycaprolactone hybrid microfluidic device using alkaline 3,5-dinitrobenzoate (DNB) is described. The reaction parameters of reagent concentration, alkalinity, and analysis time are optimized for fast reaction times and high color yield. Microfluidic devices incorporating this assay are designed and fabricated to provide a solution for low cost, point-of-care analytical applications.

**Introduction:**

- Creatinine is a small (>1 kDa) biological compound produced from creatine phosphate during energy metabolism.
- As an excretion product, creatinine is used in medical diagnostics as a baseline indicator of renal health, and as a correction factor for other more variable metabolites. [1, 2]
- Previous methods for detecting creatinine: The Jaffe (alkaline picrate) Reaction (Jaffe, 1886), Enzymatic reactions (Miller and Dubos, 1937), and UV-Absorbance (Fridolin et al., 2010). [4]
- Benedict’s (alkaline 3,5-dinitrobenzoate) method is a less common reaction that is better suited for capillary microfluidic chemistry. [5]

- Micro-Fluidic Assay Devices (µFADs) have several advantages for point-of-care (POC) vs. central laboratory testing: multiple geometries available (major limitations being designer imagination and material constraints), ease, speed, and low cost of manufacture (<$0.10/per test strip), compatibility with existing infrastructure, and use of small sample volumes (~7µL) and reagents. [3]

Hybrid paper/polymer µFADs possess the flexibility and low cost of both components. Polycaprolactone (PCL) used as the polymer component confers the added benefits of biodegradability, biocompatibility, hydrophobicity for use in channeling aqueous solutions, and customizable surface chemistry. PCL can be coated onto paper in a batch-dip process, designs can be cut into the material, and the resultant strips can be laminated together under pressure and heat to produce 3D µFADs.

**Results:**

The DNB reaction shows a high degree of promise as an assay for the detection of creatinine in a PCL hybrid microfluidic chip. The tunable nature of the reaction allows it to be customized in an application-specific format: initial results show that reaction speed is dependent on pH, while the concentration of DNB influences the intensity of the produced color.

**Conclusions and Future Work:**

A robust colorimetric assay for the determination of creatinine within the clinical range for a 24-hour urine collection (3.7-6.3 mg/dL) has been produced. [3] The assay possesses advantages over current enzymatic-based assays used in central laboratories by sacrificing a high degree of precision and low detection limit for a low cost per test, a greater ease of use, and a higher tolerance for various environmental conditions (data not shown). While this success is compelling, the refinement of the assay to increase reproducibility would allow that promising detection method to be extended to serum creatinine detection as well, which has a much lower clinical range.

As the ultimate goal of this research is to extend the analysis of creatinine to point-of-care applications, additional work must be performed in compiling individual assay components into a self-contained package. Previous research from this laboratory has explored the usage of ubiquitous smart-phone technology as a convenient platform for data capture and analysis. With this in mind, two additional goals for this research project arise:

- Creating a software algorithm for instant data analysis.
- Designing a detector (smartphone) adapter for use with the µFAD.

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**References**